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Research Article

Optimization and Characterization of Adenosine-Loaded Nanoemulsion for Enhanced Stability and Controlled Drug Release

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Abstract
Adenosine is a bioactive compound widely used in pharmaceutical and cosmetic applications due to its anti-inflammatory, wound-healing, and anti-aging properties. However, its formulation remains challenging because of its limited physicochemical stability and delivery efficiency in conventional systems. Nanoemulsions have emerged as promising delivery vehicles capable of enhancing the stability, bioavailability, and controlled release of bioactive compounds. Therefore, this study systematically investigates the influence of surfactant systems and oil phase composition on the physicochemical properties, stability, and release kinetics of adenosine-loaded nanoemulsions. Nanoemulsions were formulated using Tween 80 and Span 80 with varying hydrophilic–lipophilic balance (HLB) values and different oil phases via a low-energy emulsification method. The optimized formulation (HLB 12.86, jojoba oil) exhibited a mean droplet size of 215.7 ± 1.1 nm, polydispersity index (PDI) of 0.434, zeta potential of −54.0 ± 0.6 mV with encapsulation efficiency (%EE) of 54.7 and drug loading (%DL) of 2.8, indicating good colloidal stability. Stability studies revealed that the nanoemulsion remained stable under ambient conditions but was sensitive to temperature cycling and pH variations. *In vitro* release studies demonstrated a diffusion-controlled release mechanism best described by the Higuchi model ($R^2 = 0.842$), while the Korsmeyer–Peppas model (n = 0.638) indicated anomalous transport behavior. Cytotoxicity evaluation on Vero cells confirmed acceptable biocompatibility, with improved cell viability compared to free adenosine (IC_{50} > 100 μg/mL). Overall, this study highlights the critical role of surfactant–oil interactions in modulating nanoemulsion stability and drug release, providing insights for the rational design of delivery systems for bioactive compounds.

Keywords: Adenosine, Controlled release, Drug delivery, Hydrophilic–lipophilic balance, Nanoemulsion

1 Introduction

Adenosine (AD) is an endogenous nucleoside produced from the dephosphorylation of adenosine monophosphate (AMP) or adenosine triphosphate (ATP). It consists of an adenine moiety linked to ribose via a glycosidic bond (Figure 1) and interacts with four G protein-coupled receptors (A1, A2A, A2B, and A3) involved in various physiological processes [1]. Owing to its cardiovascular regulatory effects, AD has been widely used as an active pharmaceutical ingredient for treating cardiac arrhythmias. In cosmetics, AD acts as an anti-wrinkle agent by stimulating collagen synthesis, fibroblast activity, and skin hydration through A2A receptor activation [2-3]. However, adenosine has limited application because of its poor solubility in water, oils, and alcohols, despite being soluble in dimethyl sulfoxide (DMSO) and ammonium hydroxide (NH_4OH) [4]. Its low aqueous solubility presents significant challenges for pharmaceutical and cosmetic formulations.

Nanoemulsions have emerged as promising delivery systems for poorly soluble compounds due to their ability to improve solubility, stability, bioavailability, and permeation [5]. These systems are

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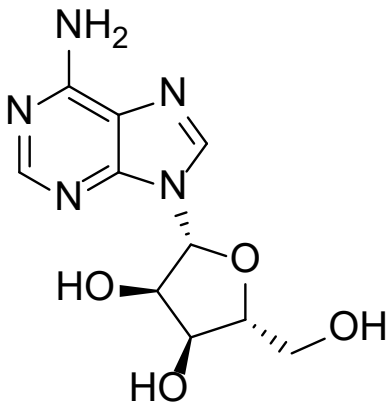

Figure 1 : Chemical structure of adenosine.

composed of oil, water, and surfactants, with droplet sizes typically ranging from 20–500 nm. Their stability and performance are strongly influenced by surfactant properties [6-7], oil phase composition [8], and surfactant hydrophilic–lipophilic balance (HLB), which affect droplet size, interfacial packing, and emulsion stability [9]. Surfactants also enhance drug solubilization by reducing interfacial tension and forming micelles above the critical micelle concentration (CMC) [10]. These advantages have led to the increasing application of nanoemulsions for the encapsulation and delivery of poorly water-soluble natural compounds and pharmaceuticals. Several nanocarrier systems, including liposomes, chitosan nanoparticles, solid lipid nanoparticles, and polymeric nanofibers, have been investigated to improve the stability, controlled release, and therapeutic efficacy of adenosine [11–15]. However, to the best of our knowledge, studies specifically investigating adenosine-loaded nanoemulsions have not yet been reported. Given the advantages of nanoemulsions for topical delivery, including enhanced stability and improved skin penetration, this study systematically evaluates the effects of surfactant hydrophilic–lipophilic balance (HLB), oil type, and surfactant composition on the physicochemical properties, stability, and release behavior of adenosine-loaded nanoemulsions.

2 Materials and methods

2.1 *Materials*

All chemicals and solvents used in this study were of analytical grade and used without further purification. Adenosine powder (purity 99%) was obtained from CHANJAO LONGEVITY CO., LTD, Myskinrecipes® (Bangkok, Thailand). Tween 80 was also purchased from CHANJAO LONGEVITY CO., LTD, Myskinrecipes® (Bangkok, Thailand). Cosmetic-grade Tween 85, Tween 60, Tween 40, Tween 20, Span 80, jojoba oil, and olive oil were supplied by S. Tong Chemicals Co., Ltd. (Nonthaburi, Thailand). Cosmetic-grade sunflower oil, coconut oil and castor oil were supplied from Krungthepchemi Co., Ltd. (Bangkok, Thailand). Ultrapure water (Type II, DI water), hydrochloric acid (Fisher Chemical™), sodium hydroxide (EMSURE®), dipotassium hydrogen phosphate (Carlo Erba™), and potassium dihydrogen phosphate (Ajax Finechem Pty Ltd., Australia) were obtained from the Department of Industrial Chemistry, Faculty of Applied Science, King Mongkut's University of Technology North Bangkok.

2.2 *Preparation of adenosine nanoemulsions using Tween surfactant*

Nanoemulsions were prepared using a low-energy emulsification method. Briefly, adenosine (1% w/w) was added to a 15 mL vial, followed by jojoba oil (1% w/w) and glycerol (2% w/w). The mixture was stirred at 60 ºC for 20 min to obtain a homogeneous oil phase. After cooling to room temperature, Tween surfactants were added at different concentrations (14, 16, 18, and 20% w/w). Deionized (DI) water was then added to reach a total weight of 10 g, and the mixture was stirred for an additional 5 min to form nanoemulsions [16]. The formulations were stored at room temperature prior to further analysis. All experiments were performed in triplicate.

2.3 *Preparation of adenosine nanoemulsions using mixed surfactants*

Nanoemulsions were prepared as described above. After formation of the oil phase and cooling to room temperature, a mixture of Tween 80 and Span 80 was added at different ratios to obtain various hydrophilic–lipophilic balance (HLB) values, calculated using Equation (1) [17].

$$HLB_{mix} = [(w_A \times HLB_A) + (w_B \times HLB_B)]/(w_A + w_B) \quad (1)$$

where HLB_{mix} is the hydrophilic–lipophilic balance of the mixed surfactant system, HLB_A and HLB_B are the HLB values of surfactants A and B, respectively, and W_A

and W_B are the corresponding weight fractions (or weights) of surfactants *A* and *B* in the mixture. Deionized water was then added to adjust the total weight to 10 g, followed by stirring for 5 min to obtain the nanoemulsion. The formulations were stored at room temperature. All experiments were conducted in triplicate. Formulation codes were assigned based on the investigated variables. F1–F8 denote formulations with different surfactant types and concentrations, F3-1 to F3-3 represent formulations with different *HLB* values, and F3jojoba–F3castor indicate formulations prepared using different oil types.

2.4 *Preparation of adenosine nanoemulsions with different oil phases*

Nanoemulsions were prepared using various oil types, including jojoba oil, olive oil, sunflower oil, coconut oil, and castor oil. Adenosine (0.1% w/w), selected oil (0.1% w/w), and glycerol (0.2% w/w) were mixed in a 15 mL vial and stirred at 60 ºC for 20 min to form a homogeneous oil phase. After cooling to room temperature, Tween 80 (18% w/w) was added as the surfactant. Deionized water was then added to reach a final weight of 10 g, and the mixture was stirred for 5 min to obtain nanoemulsions. All formulations were stored at room temperature and prepared in triplicate.

2.5 *Physicochemical characterization of adenosine nanoemulsions*

2.5.1 Particle size, polydisperse index (PDI), and zeta potential

Freshly prepared adenosine nanoemulsions were diluted with DI water at a ratio of 0.2 nanoemulsion to 40 mL DI water. The diluted samples were analyzed for particle size, polydispersity index (PDI), and zeta potential using a nanoparticle size analyzer (HORIBA Scientific, SZ-100V2) at 25 ± 0.5 ºC with a scattering angle of 90° angle [18]. All measurements were performed in triplicate.

2.5.2 Colloidal stability under storage conditions

The optimized nanoemulsion formulation was evaluated for physical stability under different storage conditions, including room temperature (30 ºC) and 4 ºC [19], under both light and dark environments. Physical appearance, particle size, and zeta potential were monitored over time.

2.5.3 Accelerated stability testing

Accelerated stability was assessed using a heating–cooling cycle test. Each cycle consisted of storing the nanoemulsion at 45 ºC for 24 h, followed by 30 ºC for 24 h [20]. A total of six cycles were performed, after which changes in physical appearance and physicochemical properties were evaluated.

2.5.4 Colloidal stability under pH conditions

The pH of the nanoemulsions was adjusted to 3, 5, 7, and 9 using 0.1 M HCl or 0.1 M NaOH. The pH was measured using a pH meter (Mettler-Toledo). Physical appearance, particle size, and zeta potential were subsequently evaluated.

2.6 *Determination of encapsulation efficiency and drug loading*

Encapsulation efficiency (*EE%*) was determined using an ultrafiltration method (Amicon® centrifugal filter unit). A 400 µL aliquot of nanoemulsion was centrifuged at 10,000 × g for 30 min at 25°C. The amount of free adenosine in the filtrate was quantified by UV–Vis spectroscopy using a previously established calibration curve. Encapsulation efficiency was calculated according to Equation (2).

$$EE(\%) = (W_t - W_f) / W_t x\ 100 \quad (2)$$

Where W_t is the total amount of adenosine initially added to the formulation, and W_f is the amount of free adenosine detected in the filtrate after ultrafiltration

Drug loading (*DL%*) was calculated as the percentage of encapsulated adenosine relative to the total carrier weight (jojoba oil, surfactants, and glycerol) according to Equation (3).

$$DL(\%) = \frac{Encapsulated\ adenosine}{Carrier\ weight} x\ 100 \quad (3)$$

2.7 *In vitro drug release*

The *in vitro* release of adenosine from nanoemulsions was evaluated using a dialysis membrane method. Briefly, 2 mL of nanoemulsion was loaded into a dialysis bag (MWCO = 7,000 Da, Solarbio) and

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Table 1: Effect of surfactant type and concentration on adenosine nanoemulsion formulation.

Formular	Surfactant	Concentration (% w/w)	HLB	Appearance
F1	Tween 80	14.0	15.0	Phase separation
F2	Tween 80	16.0	15.0	Phase separation
F3	Tween 80	18.0	15.0	Stable
F4	Tween 80	20.0	15.0	Stable
F5	Tween 85	18.0	11.0	Phase separation
F6	Tween 60	18.0	14.9	Phase separation
F7	Tween 40	18.0	15.6	Phase separation
F8	Tween 20	18.0	16.7	Phase separation

immersed in 50 mL of phosphate buffer solution (pH 6.8) under constant stirring at 100 rpm and 37 ± 0.5 ºC [20].

At predetermined time intervals (10, 20, 30, 40, 50, 60, 120, 240, 360, and 1440 min), 5 mL of release medium was withdrawn and replaced with an equal volume of fresh buffer to maintain sink conditions. The collected samples were analyzed using a UV–visible spectrophotometer at 260 nm [21].

For comparison, a control sample containing free adenosine (20 mg in 2 mL phosphate buffer, pH 6.8) was evaluated under the same conditions. All experiments were performed in triplicate.

2.8 *Cytotoxicity assay (Vero cells)*

Cell viability was evaluated using Vero cells via the MTT assay [22]. Adenosine and adenosine nanoemulsions were dissolved in dimethyl sulfoxide (DMSO) to obtain a stock concentration of 10 mg/mL and sterilized by filtration through a 0.2 µm membrane. Serial dilutions (3.125–100 µg/mL) were prepared in culture medium and incubated with Vero cells. The final DMSO concentration in all treatment wells was maintained at ≤0.1% (v/v). A corresponding vehicle control containing the same concentration of DMSO without adenosine was included to account for solvent effects, while cells exposed to 10% DMSO served as a positive cytotoxic control. Untreated cells maintained in complete culture medium served as the negative control. After 24 h of incubation, cells were treated with MTT reagent and further incubated at 37 °C for 3 h. The resulting formazan crystals were dissolved in DMSO, and absorbance was measured at 570 nm. Cell viability (%) was calculated relative to untreated control cells. Cell viability data were plotted against concentration, and the half-maximal inhibitory concentration (IC_{50}) was estimated from the dose–response relationship. When cell viability remained above 50% throughout the tested concentration range, the IC_{50} value was greater than the highest concentration evaluated (>100 µg/mL).

2.9 *Statistical analysis*

All data are presented as mean ± standard deviation (SD) (n = 3). Statistical analyses were performed using SPSS Statistics version 26.0. One-way ANOVA followed by Tukey's HSD post hoc test was used to evaluate differences in physicochemical properties under different storage conditions and pH values. Comparisons between the nanoemulsion and control formulations in the *in vitro* release study were performed using an independent Student's t-test. Statistical significance was defined as p-value < 0.05.

3 Results and Discussion

3.1 *Effect of surfactant type and concentration*

The effects of surfactant type and concentration on nanoemulsion formation were investigated (Table 1). Among all formulations, only Tween 80 at concentrations ≥18% w/w (F3 and F4) produced stable nanoemulsions without phase separation, whereas other surfactants and lower Tween 80 concentrations (<16% w/w) yielded unstable systems. This stability was attributed to the relatively high hydrophilicity of Tween 80, which effectively reduced interfacial tension and improved adenosine solubilization [23–24]. At sufficient concentrations, Tween 80 formed a dense interfacial film that stabilized droplets through steric hindrance, while insufficient surfactant caused incomplete interfacial coverage and promoted droplet aggregation [5,10]. These findings indicate that surfactant concentration plays a critical role in achieving stable nanoemulsions, even when an appropriate surfactant is selected. The influence of surfactant hydrophilic–lipophilic balance (HLB) on nanoemulsion characteristics was further investigated

in the subsequent section. Based on the stability results and the lower surfactant requirement, F3 (18% w/w Tween 80) was selected for further optimization.

3.2 *Effect of HLB*

The effect of surfactant hydrophilic–lipophilic balance (HLB) on nanoemulsion stability was evaluated using Tween 80/Span 80 mixtures (Table 2). To systematically assess the influence of HLB, formulations with HLB values both below and above 10 were investigated. Although all formulations initially formed homogeneous dispersions, systems with low HLB values (8–10) exhibited phase separation over time, indicating insufficient stabilization of the aqueous phase [25]. In contrast, the formulation with an HLB value of 12.86 (F3-1) remained stable without visible phase separation. The improved stability at HLB 12.86 was attributed to optimal surfactant packing at the oil–water interface, which reduced interfacial tension and Laplace pressure, thereby suppressing droplet coalescence [5,23]. These results indicate that an HLB value greater than 10 is necessary for stable oil-in-water nanoemulsion formation in this system, although HLB alone is not sufficient because stability is also influenced by surfactant concentration, interfacial packing, and oil phase characteristics. A similar HLB-dependent stability trend was reported by Laosinwattana *et al.,* for essential oil nanoemulsions [26], where formulations prepared at appropriate HLB values exhibited greater resistance to phase separation during storage, highlighting the critical role of HLB in maintaining nanoemulsion integrity.

3.3 *Effect of oil type*

The influence of oil phase composition on nanoemulsion formation was evaluated using different cosmetic oils (Table 3). Previous studies have reported that adenosine exhibits higher solubility in long-chain lipids [3,27]. Among the tested oils, only jojoba oil produced a stable nanoemulsion without phase separation. This enhanced stability may be attributed to its wax ester structure, which contains long-chain hydrocarbons that promote favorable interactions with surfactants and strengthen the interfacial film. In contrast, formulations prepared with olive, sunflower, coconut, and castor oils exhibited phase separation. These differences are likely related to variations in oil polarity, chain length, and viscosity, which influence interfacial packing, droplet formation, and overall nanoemulsion stability [28].

3.4 *Particle size, PDI, and zeta potential*

Adenosine nanoemulsions remained visually stable for at least 120 days at 30 °C without color change or phase separation. Selected formulations were characterized for particle size, PDI, and zeta potential (Table 4). Droplet sizes ranged from 11–215 nm, with PDI values of 0.2–0.5, indicating moderately polydisperse systems typical of low-energy emulsification [29].

The particle sizes of formulations F3 and F4 were substantially smaller than those typically reported for conventional nanoemulsions, which may be attributed to the high surfactant-to-oil ratio, resulting in the formation of surfactant-rich aggregates or micelle-like structures. Therefore, F3 and F4 were not considered optimal

Table 2: Effect of HLB.

Formulation	Tween 80 (% w/w)	Span 80 (% w/w)	Total surfactant (% w/w)	HLBmix	Appearance
F3-1	14.4	3.6	18.0	12.86	Stable
F3-2	10.8	7.2		10.72	Phase separation
F3-3	7.2	10.8		8.58	Phase separation

Table 3: Effect of oil type on the physical stability of adenosine nanoemulsions.

Formulation	Tween 80 (% w/w)	Oil type	Appearance
$F3_{jojoba}$	18	Jojoba oil	Stable
$F3_{olive}$	18	Olive oil	Phase separation
$F3_{sunflower}$	18	Sunflower oil	Phase separation
$F3_{coconut}$	18	Coconut oil	Phase separation
$F3_{castor}$	18	Castor oil	Phase separation

nanoemulsion systems due to their small particle sizes. In contrast, the incorporation of a Tween 80/Span 80 mixed surfactant system in F3-1 increased the droplet size to 215.7 ± 1.1 nm, which falls within the characteristic nanoemulsion size range while maintaining excellent physical stability. A similar droplet size range (164–210 nm) and good physical stability were reported for Tween 80/Span80-stabilized eugenol nanoemulsions [30], where oil–surfactant composition was identified as a key factor governing droplet formation and stability. These observations are consistent with the present results, highlighting the importance of surfactant organization at the oil–water interface in maintaining nanoemulsion stability.

Zeta potentials ranged from −35 to −54 mV, indicating good colloidal stability due to strong electrostatic repulsion. The optimized formulation (F3-1) exhibited a PDI of 0.43 and a zeta potential of −54.1 ± 0.6 mV, suggesting a stable dispersion despite its moderate size distribution. According to DLVO theory, a high surface charge can suppress droplet aggregation by overcoming van der Waals attraction, whereas formulations with near-neutral zeta potentials (e.g., F3, −0.2 mV) showed greater agglomeration. The optimized nanoemulsion exhibited an encapsulation efficiency (EE) of 54.7% and a drug loading (DL) of 2.8%, indicating effective incorporation of adenosine within the nanoemulsion system. The moderate EE may be attributed to the hydrophilic nature of adenosine and its partitioning within the nanoemulsion structure.

3.5 *Stability of adenosine nanoemulsions*

Formulation F3-1, which showed optimal physicochemical properties with reduced surfactant content (Table 4), was selected for stability evaluation under various conditions. At 4 °C, nanoemulsions exhibited phase separation and recrystallization within 7 days under both light and dark conditions, likely due to reduced adenosine solubility at low temperature. In contrast, samples stored at room temperature remained stable for up to 120 days without significant changes in droplet size or zeta potential, indicating minimal effect of light exposure (Figure 2).

Accelerated heating–cooling cycles induced phase separation and droplet aggregation, demonstrating poor thermal stability. This phenomenon may be explained by temperature-dependent changes in viscosity and interfacial surfactant flexibility, which promoted droplet collisions and coalescence, leading to destabilization of the nanoemulsion system [31].

The effect of pH (3–9) on nanoemulsion stability is shown in Figures 3 and 4. The nanoemulsion remained relatively stable under acidic conditions but became increasingly unstable at higher pH values, with agglomeration and phase separation observed. This behavior may be attributed to pH-dependent changes in intermolecular interactions at the oil–water interface. Although Tween 80 and Span 80 are nonionic surfactants, variations in pH can influence the hydration and conformation of the polyoxyethylene chains of Tween 80, thereby affecting interfacial packing and steric stabilization.

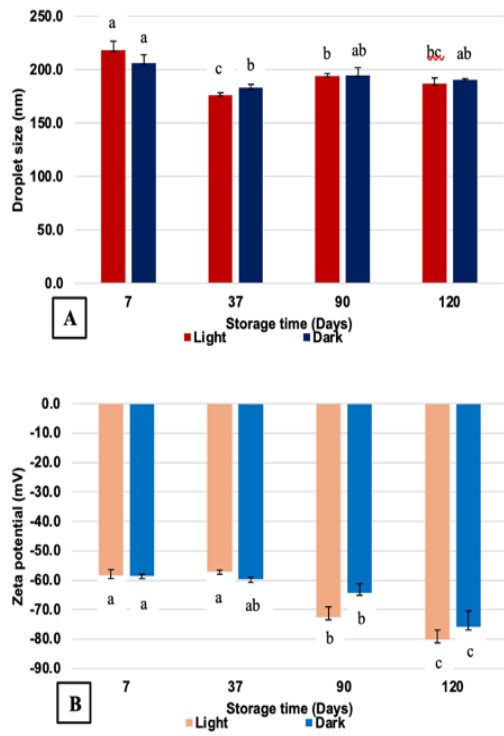

Figure 2: Changes in (A) droplet size and (B) zeta potential of formulation F3-1 during storage at room temperature under light and dark conditions. Data are presented as mean ± SD (n = 3). Different superscript letters within the same storage condition indicate statistically significant differences among storage time points, as determined by one-way ANOVA followed by Tukey's HSD post hoc test (p-value < 0.05). Groups sharing at least one common letter are not significantly different.

Table 4: Droplet size, PDI, and zeta potential of adenosine nanoemulsions.

Formulation	Tween 80 (%w/w)	Span 80 (%w/w)	Droplet size (nm)	PDI	Zeta potential (mV)
F3	18	0	12.3±1.6	0.44	-0.2±0.2
F4	20	0	11.3±0.9	0.25	-35.4±4.8
F3-1	14.4	3.6	215.7±1.1	0.43	-54.1±0.6

At higher pH values, a less effective interfacial film may promote droplet aggregation and coalescence, resulting in reduced emulsion stability. Consistent with these observations, droplet size was significantly affected by pH (one-way ANOVA, p-value < 0.001). Formulations at pH 3 and pH 7 exhibited significantly larger droplet sizes than those at pH 9 (p-value < 0.001), whereas no significant differences were observed between pH 3 and pH 7 or between pH 5 and pH 9 (p-value > 0.05). The marked reduction in droplet size at pH 9 suggests disruption of the original nanoemulsion structure and possible redistribution of formulation components following interfacial destabilization. In contrast, PDI values were not significantly affected by pH (p-value >0.05).

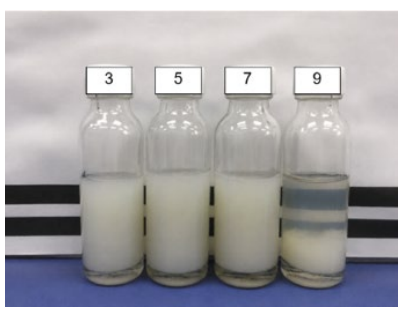

Figure 3: Visual appearance of adenosine nanoemulsions under different pH conditions (pH 3–9).

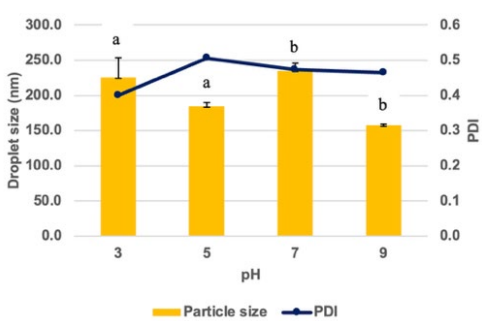

Figure 4: Effect of pH on droplet size and PDI of adenosine nanoemulsions. Data are presented as mean ± SD (n = 3). Different superscript letters indicate statistically significant differences among pH conditions as determined by one-way ANOVA followed by Tukey's HSD post hoc test (p-value < 0.05).

3.6 *In vitro drug release*

In vitro release studies (Figure 5) showed that the nanoemulsion significantly enhanced adenosine release compared with free adenosine. A biphasic release profile was observed, consisting of an initial burst release followed by sustained release, with a maximum cumulative release of 79.4%. No significant difference was detected during the first 40 min (p-value > 0.05); however, from 50 min onward, the nanoemulsion exhibited significantly greater release than the control (p-value < 0.01), with the largest difference at 1440 min (p-value < 0.001).

To elucidate the release mechanism, the release data were fitted to zero-order, first-order, Higuchi, and Korsmeyer–Peppas models (Figure 6) [32]. The Higuchi model provided the best fit (R^2 = 0.842), indicating diffusion-controlled release, while the Korsmeyer–Peppas model (R^2 = 0.839, n = 0.638) suggested anomalous transport involving both diffusion and interfacial relaxation processes. Similar findings were reported by Adam *et al.,* [16] for a Zanthoxylum rhetsa nanoemulsion, in which the Higuchi model also provided the best fit, indicating diffusion-controlled release as the predominant release mechanism.

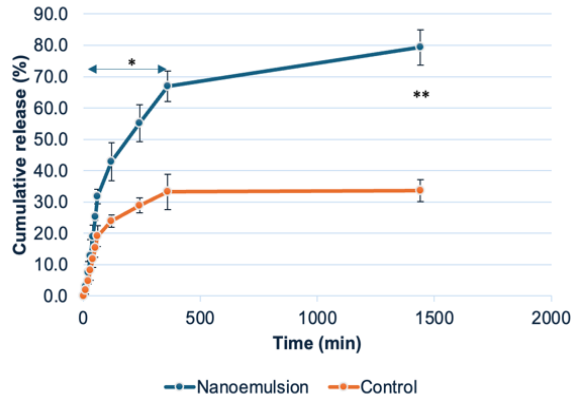

Figure 5: Comparative *in vitro* release profiles of adenosine from the nanoemulsion and free drug (control). Data are presented as mean ± SD (n = 3). * p-value < 0.01 and ** p-value < 0.001 compared with the control formulation at the corresponding time point.

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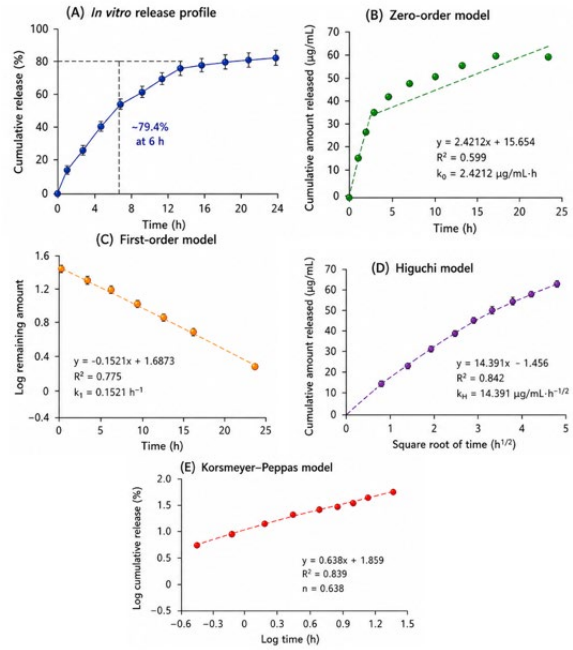

Figure 6: Kinetic modeling of in vitro adenosine release from nanoemulsion: (A) cumulative release (%) as a function of time; (B) zero-order model; (C) first-order model; (D) Higuchi model; and (E) Korsmeyer–Peppas model.

It should be noted that the dialysis membrane may introduce an additional diffusion barrier; therefore, the measured release profiles reflect both drug release from the nanoemulsion and diffusion through the membrane. Nevertheless, because both formulations were evaluated under identical conditions, the method remains suitable for comparative assessment of release behavior. These results demonstrate the potential of nanoemulsions to improve the controlled delivery of adenosine.

3.7 *Cytotoxicity assay*

The cytotoxicity of adenosine nanoemulsion and free adenosine was evaluated in Vero cells over a concentration range of 3.125-100 μg/mL, and cell viability was expressed as a percentage relative to untreated controls (Figure 7). The nanoemulsion exhibited cell viability ranging from 64.2% to 81.8%, whereas free adenosine exhibited slightly lower viability values ranging from 60.6% to 79.5%.

At the highest tested concentration (100 μg/mL), the nanoemulsion maintained marginally higher cell viability (81.8 ± 1.2%) than free adenosine (79.5 ± 1.7%). Both formulations showed acceptable cytocompatibility, with cell viability remaining above 60% [33] across all tested concentrations and IC_{50} values exceeding 100 μg/mL. The slightly higher viability observed for the nanoemulsion may be attributed to the encapsulation of adenosine within dispersed droplets, which can reduce direct exposure of cells to free adenosine and modulate drug–cell interactions. These findings suggest that the developed nanoemulsion maintains an acceptable safety profile in vitro and may serve as a promising delivery platform for adenosine in topical or pharmaceutical applications. However, further studies using skin-relevant cell models are recommended to confirm its suitability for topical delivery.

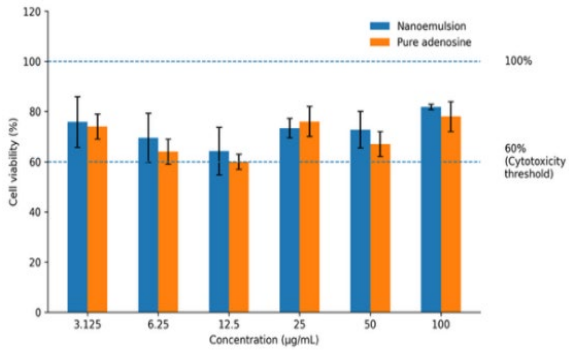

Figure 7: Cell viability of Vero cells treated with adenosine nanoemulsion and free adenosine at different concentrations. Data are presented as mean ± SD (n = 3).

4 Conclusion

This study successfully demonstrated that a nanoemulsion system can enhance the solubility and release behavior of adenosine. The findings highlight the critical roles of surfactant composition and oil phase selection in governing nanoemulsion formation, stability, and performance.

The optimized formulation (F3-1), composed of Tween 80, Span 80, and jojoba oil, provided favorable conditions for adenosine incorporation, likely due to enhanced intermolecular interactions at the oil–surfactant interface. This formulation exhibited a nanoscale droplet size, good physical stability for up to 120 days at room temperature, resistance to light-induced degradation, and acceptable cytocompatibility in Vero cells. In vitro release studies demonstrated an initial burst release followed by sustained diffusion-controlled release, indicating its potential to improve adenosine delivery.

Overall, this work provides valuable insight into the relationship between formulation parameters and nanoemulsion performance and establishes a rational strategy for developing stable and efficient delivery systems for hydrophilic bioactive compounds such as adenosine. Future studies should further investigate skin permeation and in vivo performance to validate the applicability of the developed nanoemulsion for pharmaceutical and cosmeticeutical applications.

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Author Contributions

N.T. : Investigation, Methodology, Writing-original draft. K.P.: Investigation. S.T.: Formal analysis, Validation, Writing-review and editing. L.S.: Validation, Writing-review and editing. S.S.: Validation, Writing-review and editing. I.S.: Validation, Writing-review and editing. N.T.*: Conceptualization, Methodology, validation, Writing-review and editing, Supervision. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors utilized the ChatGPT tool to enhance the language and readability of the manuscript.

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