



Production of liposome loaded with nicotinamide mononucleotide using Taylor-Couette reactor

Isu Kim , Beom Soo Kim ^{*}

Department of Chemical Engineering, Chungbuk National University, Cheongju, Chungbuk 28644, Republic of Korea

ARTICLE INFO

Keywords:

Nicotinamide mononucleotide
Liposome
Taylor-Couette reactor

ABSTRACT

Nicotinamide mononucleotide (NMN) is recognized for its potential in resisting age-related diseases, metabolic disorders, and neurodegenerative conditions. However, challenges persevere in ensuring NMN's stability, bioavailability, and efficient delivery to target tissues. This study targeted to overcome these challenges by preparing NMN-loaded liposomes using a Taylor-Couette reactor, a novel approach offering precise control over flow conditions and enhancing reproducibility and scalability. The average size of liposomes decreased from 184.9 nm to 125.2 nm as the rotation speed increased from 600 to 1500 rpm. Based on average zeta potential values, stable liposomes were prepared at 750–1500 rpm. These liposomes were preserved more than 80 % for 180 days. *Ex vivo* tests using Franz cell diffusion confirmed the efficiency of NMN-loaded liposomes in permeating the skin. The cumulative release percentage of liposomes over porcine skin was 96.8 % in 12 h. Overall, this study proposes valuable intuitions into the preparation and depiction of NMN-loaded liposomes using novel approaches, offering auspicious prospects for biomedical applications.

1. Introduction

Nicotinamide mononucleotide (NMN), as a precursor to nicotinamide adenine dinucleotide (NAD^+), is gaining importance due to its physiological functions within the body. [1,2] NAD^+ plays crucial roles in energy metabolism, DNA repair, gene expression regulation, and cellular signaling pathways. [3] Recently, low NAD^+ levels were linked to multiple disease states, including metabolic and neurodegenerative diseases. Lower NAD^+ levels are now also known to correlate with ageing in rodents and humans. [4–6] NMN can be converted into NAD^+ through the action of nicotinamide mononucleotide adenylyl transferase. [7] Research has shown that NMN supplement can increase cellular NAD^+ levels, thus potentially justifying age-related decay in physiological functions and promoting overall health span. [2] NMN supplementation upregulates the expression of platelet-derived growth factor receptor beta and other genes involved in muscle remodeling. As a result, NMN enhances muscle insulin sensitivity, insulin signaling, and remodeling in overweight or obese women with prediabetes. [8] Fig. 1 (a) illustrates the role of NMN upon entering the body and its function within the plasma membrane. Moreover, NMN has demonstrated effects in various preclinical studies, including enhancing mitochondrial function, improving insulin sensitivity, and protecting against age-related

cognitive decline. [4,9].

NMN can be synthesized by converting nicotinamide (NAM) with the assistance of nicotinamide phosphoribosyltransferase (NAMPT) through the salvage pathway. Recombinant *Escherichia coli* strains, modified to include the NAMPT gene along with phosphoribosyl pyrophosphate synthetase 1 (PRPS1) and PRPS2 (from *Homo sapiens*) genes, enhanced NMN production. The metabolically engineered *E. coli* BL21(DE3) strain has the capability for intracellular NMN production from inexpensive substrates such as glucose, lactose, and NAM. The BL21(DE3) strain produced a maximum concentration of 2.31 mM NMN during batch fermentation when supplemented with 1 % ribose, 1 mM Mg^{2+} , 1 mM phosphate, 1 % lactose as an inducer, and 0.5 % nicotinamide. [10] Genetically modified *E. coli* can be grown using either batch or fed-batch methods. To achieve high NMN productivity, studies using the fed-batch method have been mainly conducted. Various fed-batch feeding strategies include linear feeding, exponential feeding of the co-substrate (glucose) and continuous feeding of the substrate (NAM), and pH-stat glucose feeding and continuous feeding of NAM. [11–13] Currently, NMN production via pH-stat fed-batch fermentation demonstrated the highest productivity at 1.13 g/L/h. The NMN produced was purified using size exclusion chromatography. [13]

Despite the growing interest in NMN, challenges continue about its

^{*} Corresponding author.

E-mail address: bskim@chungbuk.ac.kr (B.S. Kim).

<https://doi.org/10.1016/j.cej.2024.158703>

Received 13 August 2024; Received in revised form 11 December 2024; Accepted 16 December 2024

Available online 17 December 2024

1385-8947/© 2024 Elsevier B.V. All rights are reserved, including those for text and data mining, AI training, and similar technologies.

bioavailability, stability, and delivery to target tissues. Bioavailability refers to the extent to which NMN is administered through dermal administration, reaches the dermal layer, and is reliably delivered to the capillaries, where it can be converted to NAD^+ . [14,15] The main issue with currently available oral NMN capsules is that they are primarily metabolized in the stomach and liver, leading to their excretion as NAM. To address this, our research aims to stabilize the molecular structure of NMN through liposomal formulation and utilize transdermal delivery for effective administration. Liposomes, nano-sized lipid vesicles, represent a versatile platform in drug delivery owing to their ability to encapsulate both hydrophilic and hydrophobic compounds. [16] Through transdermal delivery, NMN is expected to be directly absorbed into the bloodstream, enhancing its bioavailability and reducing losses during the metabolic process. [17] In addition, to reach the dermal layer, NMN must pass through the stratum corneum and epidermis.

Since NMN is a highly hydrophilic substance making it difficult to pass through the stratum corneum and epidermis to reach the dermal layer, we aimed to encapsulate NMN in a phospholipid bilayer. [18]

The purity of NMN was reported to decrease to 65.7 % when stored at 25°C for 30 days, raising concerns about its stability. [19] The mechanism of NMN degradation as it reacts with water in aqueous solution can be observed in Fig. 1(b). In response to these challenges, we have opted for the adoption of liposomes as a resolution, specifically for their potential in providing physicochemical stabilization. However, NMN's strong hydrophilic nature complicates its encapsulation using conventional methods, such as thin-film hydration, reverse-phase evaporation, or extrusion, exacerbating issues related to liposome formation. Moreover, conventional methods often produce liposomes with insufficient size uniformity needed for precise control of drug delivery rates, and the use of organic solvents in liposome preparation tends to leave residual

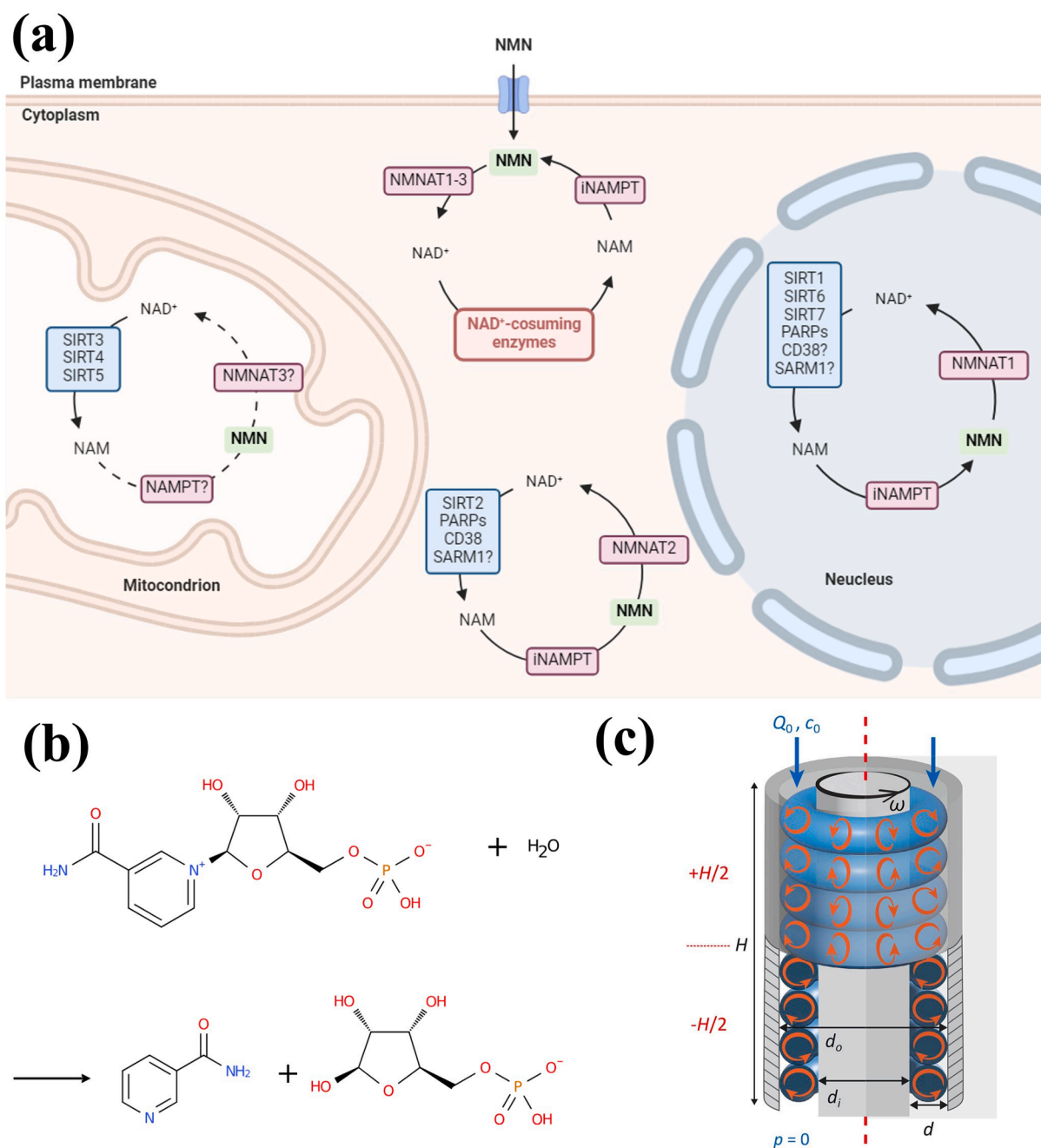


Fig. 1. (a) Effects of NMN in various sites within the body, (b) mechanism of NMN decomposition into NAM in solution, (c) schematic diagram of Taylor-Couette reactor. [83].

solvents that can pose toxicity risks and degrade active ingredients. [20–22] Consequently, the scalability and stability of liposome production processes are compromised, impeding their extensive adoption in industrial and point-of-care applications. This limitation underscores the need for novel approaches to overcome the shortcomings of traditional liposome formation techniques.

In this context, this study aims to investigate the production of NMN-loaded liposomes using a Taylor-Couette reactor (TCR), aiming to improve NMN delivery efficiency and bioavailability. TCR is a distinct type of reactor that utilizes the Taylor-Couette flow phenomenon. [23] This reactor consists of a cylindrical shell with a rotating inner cylinder, creating an annular gap where various flow regimes can be formed. Fig. 1(c) depicts a schematic diagram of the apparatus used in this experiment. The mixing conditions within TCR can be adjusted independently from the axial flow by changing the rotational speed of the cylinders and the reactor's geometry. [24] This flexibility allows for tailored flow conditions, ranging from high shear forces for mixing and dispersing to high flow segregation resulting in plug-flow behavior. Firstly, it provides a precisely controlled environment, enhancing reproducibility and scalability. The controlled flow conditions ease uniform mixing and dispersion of NMN within the lipid bilayers, resulting in liposomes with improved encapsulation efficiency. Encapsulation efficiency can be defined as the amount of drug loaded inside and outside the phospholipid bilayer of liposomes. [25,26] Additionally, the unique flow patterns enable efficient interaction between the NMN solution and lipid components, promoting rapid and uniform encapsulation. In using a TCR, fluid flow and shear forces can be finely tuned, enabling the production of uniform and stable liposomes, thus addressing the structural instability of NMN and preventing its degradation into NAM. [27] Moreover, uniform and small-sized particles enhance skin permeability, facilitating NMN's penetration into the dermal layer and potentially distributing its anti-aging effects and other physiological benefits throughout the body. The reactor's versatility allows for optimization of flow conditions to achieve desired liposome characteristics. TCR presents a promising approach to overcome limitations in liposome preparation for NMN delivery. Producing NMN-loaded liposomes using TCR is simpler than conventional methods, making large-scale production easier.

The prepared NMN-loaded liposomes were characterized for their physicochemical properties and *ex vivo* pig skin penetration test using Franz cell diffusion, laying the foundation for potential biomedical applications. Overall, understanding the production and characterization of NMN-loaded liposomes can contribute to the development of novel delivery systems for NMN, thereby advancing its therapeutic potential in various health conditions. This study is the first report proposing a method for preparing NMN-loaded liposomes using TCR.

2. Materials and methods

2.1. Chemicals

NMN (CAS No. 1094–61-7, 99.97 %) was obtained from EffePharm Co., China. Phosphatidylcholine (CAS No. 97281–47-5, 75 %, Soya SPL 75H) was sourced from Neuropid, Korea. [28] Octanoic acid (CAS No. 124–07-2) was acquired from Earthborn, Germany. Alkyl polyglucoside (CAS No. 68515–73-1, TRITON™ CG-110) was obtained from Sigma-Aldrich. PBS 01 buffer (pH 7.4) was obtained from SolBio Corp., Korea.

2.2. Materials

The Franz cell membrane (porcine skin, 2 cm x 2 cm x 400 μm) was bought from Apures, Korea. A standard jacketed Franz cell (8 mL) was sourced from BNC Tech, Seoul, Korea. Polytetrafluoroethylene cylindrical magnetic bar (28 × 8 mm, 8 × 3 mm) was obtained from Cowie, UK.

2.3. Equipment

The Taylor-Couette reactor (LCTR®–Lab II) used in this study was supplied by Laminar, Korea. The volume of the TCR used in this experiment is 200 mL, with an outer cylinder diameter of 60 mm and a rotating inner cylinder diameter of 50 mm.

2.4. Preparation of liposome using TCR

To prepare liposomes, powdered phosphatidylcholine (6.0 g), which acts as the phospholipid bilayer and a lipophilic surfactant, was dissolved in 1 mL of octanoic acid and heated to 65°C. Concurrently, distilled water and octanoic acid were also heated to 65°C. Following this, 2 g of NMN was dissolved in 30 mL of the heated distilled water. [29] In a 200 mL TCR, the lecithin-octanoic acid mixture, 1 mL of alkyl polyglucoside, octanoic acid (20 mL), and 5 min of reaction time were employed to ensure proper dispersion of hydrophilic surfactants, such as phosphatidylcholine, on the oil phase. [30,31] Subsequently, the aqueous phase containing dissolved NMN was added to the reaction vessel, forming a water-in-oil (W/O) emulsion. Following a 30-min incubation period, 148 mL of distilled water was introduced to the W/O emulsion, creating a water-in-oil-in-water (W/O/W) emulsion. [30,32] The reaction vessel was maintained at 65°C for 3 h while subjecting the mixture to various rotational speeds to facilitate liposome formation. Sampling was conducted every 0.5 h to observe the difference in liposome particle size over time at 1000 rpm and 1500 rpm.

2.5. Preparation of liposome using flask

To evaluate the liposome production capability influenced by the strong shear stress of TCR, liposomes were prepared in a flask for comparative analysis. The procedure is as follows: First, 6.0 g of powdered phosphatidylcholine was dissolved in 1 mL of octanoic acid and heated to 65°C. Simultaneously, distilled water and octanoic acid were also heated to the same temperature. Next, 2 g of NMN was dissolved in 30 mL of the heated distilled water. [29] In a 250 mL flask, the lecithin-octanoic acid mixture was combined with 1 mL of alkyl polyglucoside and 20 mL of octanoic acid, allowing for 5 min to ensure adequate dispersion of hydrophilic surfactants, such as phosphatidylcholine, within the oil phase. Following this, the aqueous phase containing the dissolved NMN was added to the flask, resulting in the formation of a W/O emulsion. [30,31] After a 30-min incubation, 148 mL of distilled water was introduced to the W/O emulsion, yielding a W/O/W emulsion. [30,32] The flask was maintained at 65°C for 3 h while subjecting the mixture to various rotational speeds to facilitate liposome formation.

2.6. Verification of the stability of NMN-loaded liposomes

To observe NMN in aqueous solution and liposomes produced at various rpm through TCR, samples were stored at room temperature for 30 days. Subsequently, samples were analyzed by HPLC. This experiment was performed in triplicate. The percentage of remaining NMN was calculated as:

$$\text{Remaining NMN}(\%) = \frac{\text{Amount of NMN in solution after 30 days}}{\text{Amount of NMN in solution before storing}} \times 100$$

2.7. Ex vivo test of NMN-loaded liposomes using Franz cell diffusion method

To assess the release of NMN from liposomes prepared using a TCR at 1000 rpm, 1.5 mL of a 1 % liposomal dispersion solution of NMN was added to the donor compartment of an 8 mL Franz cell diffusion device. The Franz cell held prepared PBS buffer and a porcine skin membrane serving as the biological barrier. A pig skin membrane was carefully

positioned between the donor and acceptor compartments of the Franz cell. The temperature was maintained at $37 \pm 0.5^\circ\text{C}$, and the rotation speed was set to 300 rpm using a magnetic stirrer. [33] Sampling of the PBS solution containing released NMN was conducted at specific time intervals, with 1 mL samples collected and replaced with fresh medium each time to maintain volume consistency. Sampling was performed through the side arm of the cell using a long syringe. All procedural steps were executed under sterile conditions to prevent contamination and ensure the integrity of the experiment. [34]

2.8. Analytical methods

All samples underwent analysis using high-performance liquid chromatography (HPLC) performed on a YL 9100 system (Younglin Inc., Korea). Prior to analysis, all solutions were filtered using a $0.22\ \mu\text{m}$ membrane. NMN concentration was quantified using an Optima Pak C18 column ($250 \times 10\ \text{mm}$, RS Tech, Korea) at a column temperature of 20°C . Isocratic elution was performed using a mobile phase consisting of acetonitrile (10 %, v/v) and water (90 %, v/v) at a flow rate of 1.0 mL/min. The NMN peak was detected at 260 nm using a UV-vis detector. [27,35] The zeta potential and particle size were measured using a Nano Particle Tracking Analyzer model PMX 430, manufactured by Particle Metrix, Korea. Cryo-transmission electron microscopy (Cryo-TEM) was performed using a model Krios G4 Cryo-TEM from Thermo Fisher Scientific. The UV-vis spectrophotometer used for turbidity analysis is the Mega-U600 (Scinco, Korea).

2.9. Statistical analysis of experimental data

To derive meaningful differences from the experimental data, an F-test was conducted to assess the equality of variances. Based on the resulting F-value, if ($p < 0.05$), a two-sample t-test for unequal variances was performed; conversely, if ($p > 0.05$), a two-sample t-test for equal variances was employed. Statistical significance was determined with ($p < 0.05$) indicating a statistically significant difference, while ($p > 0.05$) suggested no significant difference. This rigorous statistical analysis was carried out in accordance with established methodologies in the literature, ensuring the reliability and validity of our findings. [36,37]

3. Results and discussion

3.1. Computational fluid dynamics (CFD) simulation of TCR

The governing incompressible continuity and momentum equations (Navier-Stokes equation) are:

$$\nabla \cdot \mathbf{u} = 0$$

$$\rho(\mathbf{u}' + (\mathbf{u} \cdot \nabla)\mathbf{u}) = -\nabla p + \nabla \cdot (\mu(\nabla\mathbf{u} + \nabla\mathbf{u}^T)) + \mathbf{F}$$

where $\mathbf{u}$, ρ , p , and ν denote velocity, density, pressure, and kinematic viscosity of the fluid, respectively. [38]

For boundary coefficient and azimuthal velocity of flow,

$$u_r = u_z = 0, u_\theta = -\frac{\omega \cdot r_i^2}{(r_o^2 - r_i^2)} \cdot \mathbf{r} + \frac{\omega \cdot r_o^2}{(r_o^2 - r_i^2)} \cdot \frac{1}{r} \quad (1)$$

where r and θ represent radial and azimuthal directions of the cylindrical coordinate system, respectively. The angular velocity of the inner cylinder is denoted by ω ; the inner and outer radius are represented by r_i and r_o , respectively. It should be noted that equation (1) is the solution under the idealization of infinitely long cylinders where endwall effects are ignored; this solution is not realized exactly in an physical experiment or simulation that include endwall effects. [39,40] Boundary condition is $u_\theta = 0$ for every wall except inner cylinder wall.

When inputting into the CFD provided by the MATLAB app FEATool Multiphysics v1.16 (<https://www.featool.com>), it is possible to

determine the fluid flow inside the TCR and the resulting shear stress. The input constants are as follows: the density and viscosity are measured to be $1.0289\ \text{g/cm}^3$ and $0.798\ \text{cP}$, respectively, and the angular velocities for each rpm are shown in Table 1.

Fig. 2(a) shows the velocity field of the symmetrical flow as a function of rpm. This figure illustrates when the Taylor-Couette flow begins to form at different rpm values. The left side of each figure represents the rotating inner cylinder, while the right side represents the stationary outer cylinder. [41–43] To prepare NMN-loaded liposomes, it is essential to eliminate the interface between polar NMN solution and non-polar octanoic acid to form a single phase. This process requires well-dispersed surfactants and strong shear forces. In a TCR, toroidal vortices enhance radial mixing, preventing phase segregation and coagulation, while facilitating strong shear stress and efficient material transfer in all axial directions. [44] This results in uniform and consistent liposomes. Phosphatidylcholine disperses, allowing the lipophilic portion to combine with octanoic acid, while the hydrophilic portion interacts with NMN solution, leading to stability. Up to 600 rpm, fluid flow occurs only in the vicinity of the inner cylinder, leading to a concentration of shear stress at the end of the cylinder where fluid velocity at the wall is zero. At 650 rpm, perpendicular flow to the axis begins to emerge. At 700 rpm, the Taylor-Couette flow further develops, accompanied by a decrease in the number of Taylor vortices. Beyond 750 rpm, the flow stays in the Taylor-Couette regime, with an increase in shear stress inside as the rpm rises, and a tendency for the number of Taylor vortices to decrease. At 750 rpm, there were 22 rings, which decreased to 19 rings at 1500 rpm. Additionally, in the velocity field, the red areas indicate higher speeds, while the gradually decreasing blue areas represent lower speeds. This observation suggests that as the rotational speed increases, the flow velocity of the fluid also increases, leading to faster and more vigorous mixing of the oil and aqueous phases. Fig. 2(b) presents the shear stress field of the symmetrical flow as a function of rpm. This figure shows how the forces are applied within the TCR depending on the rpm. Near the left side of the figure, which corresponds to the rotating inner cylinder, small shear stresses occur, while larger shear stresses are observed at the right side, where the stationary outer cylinder is located, indicating a tendency for the fluid to flow from the outside to the inside. [45] Similarly, at 600 rpm, the pressure remains constant in the z-axis direction, but it begins to change shape starting from 650 rpm due to the Taylor-Couette flow. At 700 rpm, a developed pressure field is observed, albeit weakly. By 750 rpm, the pressure field is fully developed. As the rpm increases, the pressure difference also increases, indicating that the fluid is mixed more rapidly and vigorously. [46] The strong shear stress within the fluid eases rapid and uniform formation of liposomes, enhancing encapsulation efficiency compared to conventional methods. Moreover, stable liposomes contribute to maintaining the stability of NMN molecules inside the liposome in an otherwise unstable aqueous environment.

The Taylor number (Ta) is a dimensionless number that is used to characterize the flow regime in rotating systems. It is defined as the ratio of inertial forces to viscous forces and is a critical parameter in determining the flow. Ta is expressed as:

$$Ta = \left(\frac{r_i \omega d}{\nu} \right) \left(\frac{d}{r_i} \right)^{1/2} \quad (2)$$

where d represents the width between the inner and outer cylinders. If the rpm increases excessively, resulting in the size of the vortex becoming smaller than d , the flow of the fluid will become unstable. In

Table 1

Conversion of angular velocity (ω) and Taylor number (Ta) with respect to rpm.

rpm	600	650	700	750	1000	1500
ω (rad/s)	62.832	68.068	73.304	78.540	104.72	150.08
Ta	4401.5	4768.3	5135.1	5501.9	7335.9	10513

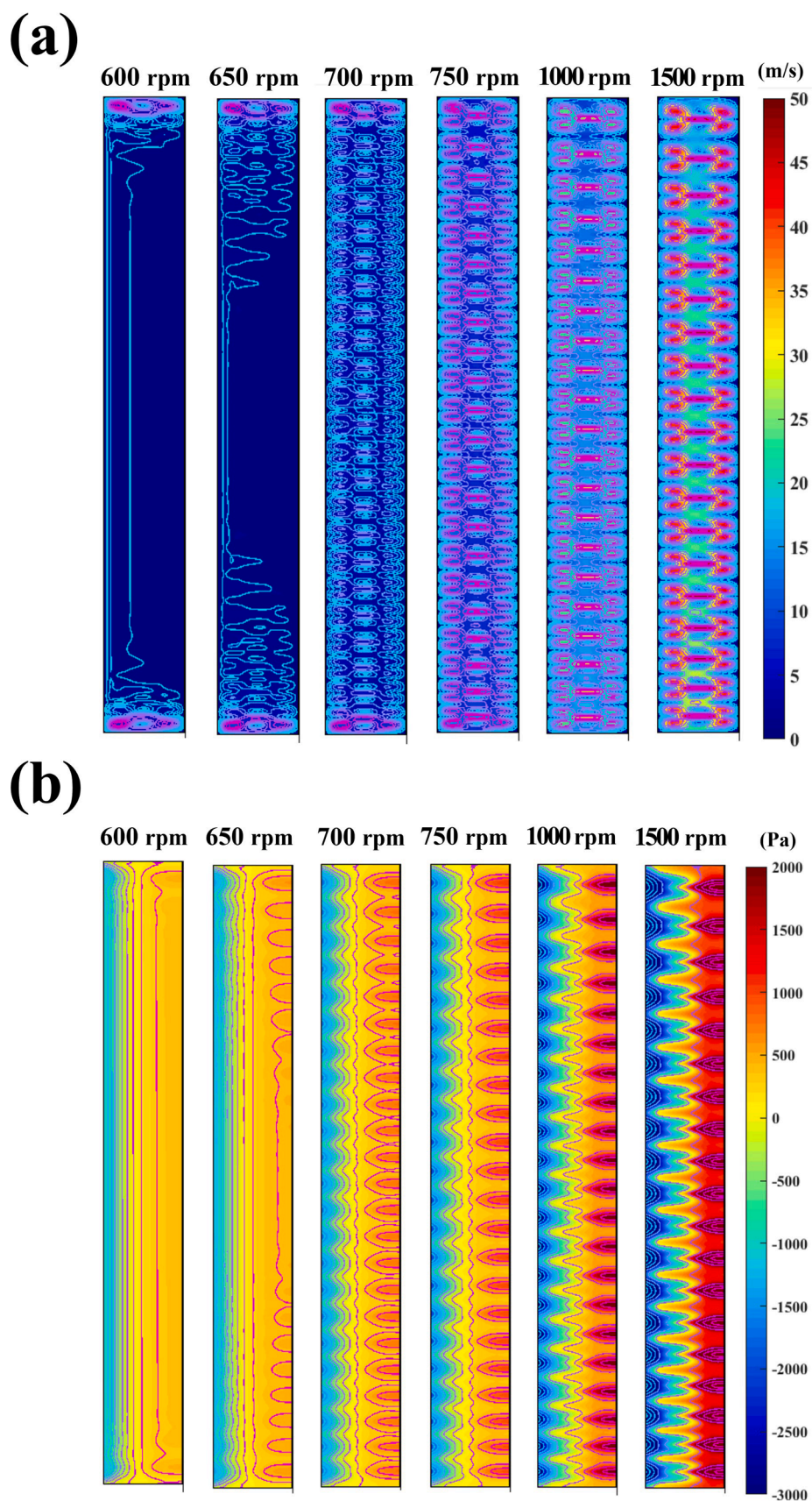


Fig. 2. Taylor-Couette reactor modeled with CFD. (a) velocity field (m/s), (b) shear stress field (Pa).

the context of turbulent vortex flow, this instability leads to the cessation of vortex formation, and the fluid flow transitions into a state of turbulent flow. This transition is significant because it has direct implications for the behavior of the fluid, particularly in processes that rely on the formation of structures such as liposomes. The formation of liposomes is highly sensitive to the conditions of the fluid environment, including shear stress. Kataoka et al. [47] showed when shear stress decreases due to the transition to turbulent flow, the ability of the fluid to facilitate the proper assembly of lipids into liposomes is compromised. This can result in lower yields of liposome formation or the production of liposomes with undesirable characteristics, such as size or stability issues. [48] Specifically, a Taylor number greater than 15,000 is typically indicative of turbulent flow conditions. In the context of this experiment, the maximum rpm of 1500 results in a Ta of 10,513. This value suggests that the flow is still within the turbulent vortex flow regime, but it is approaching the threshold where turbulence may become more pronounced. The transition from turbulent vortex flow to turbulent flow, driven by excessive rpm and reduced vortex size, can have significant consequences for processes reliant on specific fluid behaviors. [47]

3.2. Characterization of NMN-loaded liposomes prepared using TCR

The difference between liposomes prepared using TCR and those produced via magnetic stirring in flasks can be visually compared in Fig. 3(a). The color of the dispersion solution shows how well the hydrophilic surfactant, phosphatidylcholine, has been emulsified. A darker color suggests inadequate emulsification of phosphatidylcholine, while a lighter color shows stable emulsification. Liposomes produced in flasks under the same rpms but with the use of a magnetic stirrer show a darker color in the dispersion solution, indicating inadequate emulsification of phosphatidylcholine. At 600 rpm and 650 rpm, phosphatidylcholine aggregated due to inadequate shear force. Despite repeated experiments, phosphatidylcholine continued to aggregate and become isolated in the reactor. From 700 rpm onwards, isolated phosphatidylcholine was not observed in the reactor. Nevertheless, compared to liposomes prepared in flasks, those produced in the reactor show a lighter color, indicating greater stability. From 750 rpm onwards, the Taylor-Couette flow fully develops, forming stable liposomes, as shown by the lighter color of the dispersion solution.

To analyze the trends observed visually in more detail, turbidity analysis was conducted, and the results are shown in Fig. 3(b). A higher turbidity indicates that emulsification occurred more stably. [49] Therefore, optical density (OD) at 600 nm was measured using a UV-vis spectrophotometer. As a result, both liposomes created in the flask and those made with TCR showed an increasing trend in turbidity as the rpm increased. As confirmed by CFD, at 600 rpm, where axial flow is the main flow, the OD was lower than that of the flask.

However, starting from 650 rpm, where Taylor-Couette flow begins to form, the OD in TCR was higher. This indicates that the formation of Taylor-Couette flow leads to the fluid rotating at a high speed, resulting in shear stress that initiates the formation of liposomes. At 700 rpm, where Taylor-Couette flow is fully developed according to CFD, the OD in the flask differs by more than six times. Therefore, while the OD increases with rising rpm in both the flask and TCR, the OD in TCR shows a sharp increase between 650 rpm and 700 rpm, which is attributed to the formation of Taylor-Couette flow.

Cryo-TEM is a powerful tool for understanding the morphology and structure of amphiphilic molecules, particularly liposomes, as well as their interactions with surfactants in aqueous solutions. [50] These images were captured to confirm the shape and bilayer structure of liposomes, with the results presented in Fig. 4. [51] First, examining the shape of the particles created at 600 rpm where Taylor-Couette flow was not formed, the particles are large and contain smaller particles within them, indicating that complete liposomes were not formed. This structure resembles a W/O/W emulsion rather than the desired vesicle form.

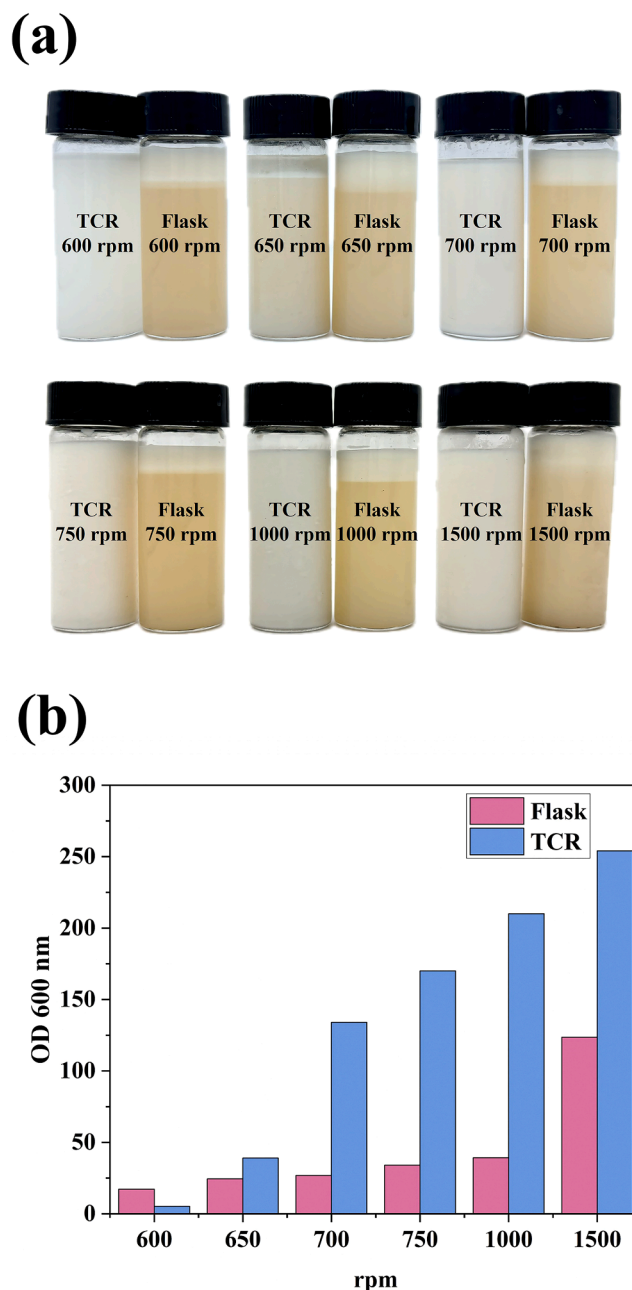


Fig. 3. Comparison of liposomes produced using TCR and flask according to rpm change. (a) visual image, (b) turbidity (optical density at 600 nm).

Such a structure is highly unstable, leading to particle collapse, and the large size of the particles prevents them from passing through the stratum corneum and epidermis, making drug delivery through the skin difficult. In the Cryo-TEM images at 650 rpm, the particles appear to be clustered together rather than separated. This state results from attractive forces between the particles, causing them to aggregate. This appearance is not only unstable but also fails to structurally stabilize NMN. At 700 rpm, the particle size becomes relatively smaller, and the aggregation phenomenon disappears; however, the particles are not round and show an uneven shape. It is still evident that a W/O/W emulsion form is maintained. Non-uniform particles lead to an inconsistent water phase within the liposomes, resulting in decreased encapsulation efficiency. [20] From 750 rpm onwards, particle aggregation is prevented, allowing for the stable formation of liposomes. Notably, at 1000 rpm and 1500 rpm, the uniformity of the particles improved compared to 750 rpm, indicating that liposomes were formed

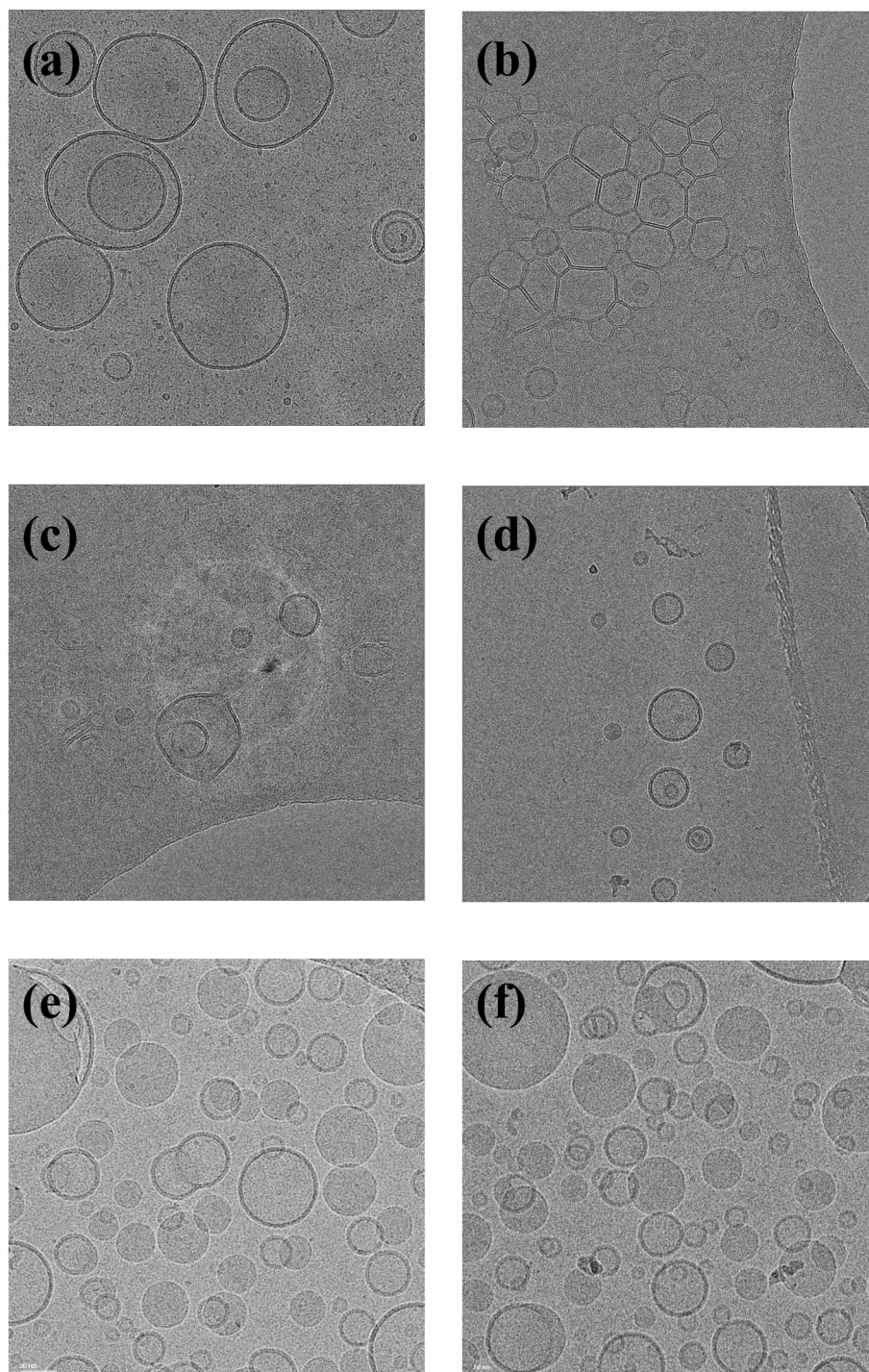


Fig. 4. Liposome loaded with NMN prepared by TCR observed with the Cryo-TEM. (a) 600 rpm, (b) 650 rpm, (c) 700 rpm, (d) 750 rpm, (e) 1000 rpm, (f) 1500 rpm. (a)-(d) 47000x, (e), (f) 65000x.

in a vesicle shape rather than a W/O/W emulsion. These images confirm the bilayer structures of the liposomes. This structure, due to its small particle size, can pass through the stratum corneum and epidermis. Additionally, the consistent size ensures high encapsulation efficiency, which allows NMN to be encapsulated in liposomes for stabilization. Since the particles are all separated, they can be stored stably at room temperature without aggregation. [52] Under the same rpm, when comparing liposomes produced in a magnetic stirrer, the differences become even more pronounced. Fig. 5 shows the Cryo-TEM images of liposomes produced in a flask. The liposomes produced at 600 rpm are irregularly shaped, non-uniform, and large. They also appear to be

merged or on the verge of merging, indicating that they are not stable liposomes. When the size of the liposome particles increases, they cannot pass through the stratum corneum and epidermis, remaining on the surface of the skin. Additionally, if the particles merge and increase in size, the loaded NMN cannot be stored stably. The liposomes produced at 650 rpm also exceed 1 μm in size, making it difficult for them to penetrate the skin barrier and reach the dermis layer. Furthermore, their shapes are not round, indicating instability. At 700 rpm and 750 rpm, the shapes become relatively round, but they are still large and inconsistent, resulting in lower efficacy compared to liposomes made with TCR. At 1000 rpm, smaller liposomes are observed, but unstable large

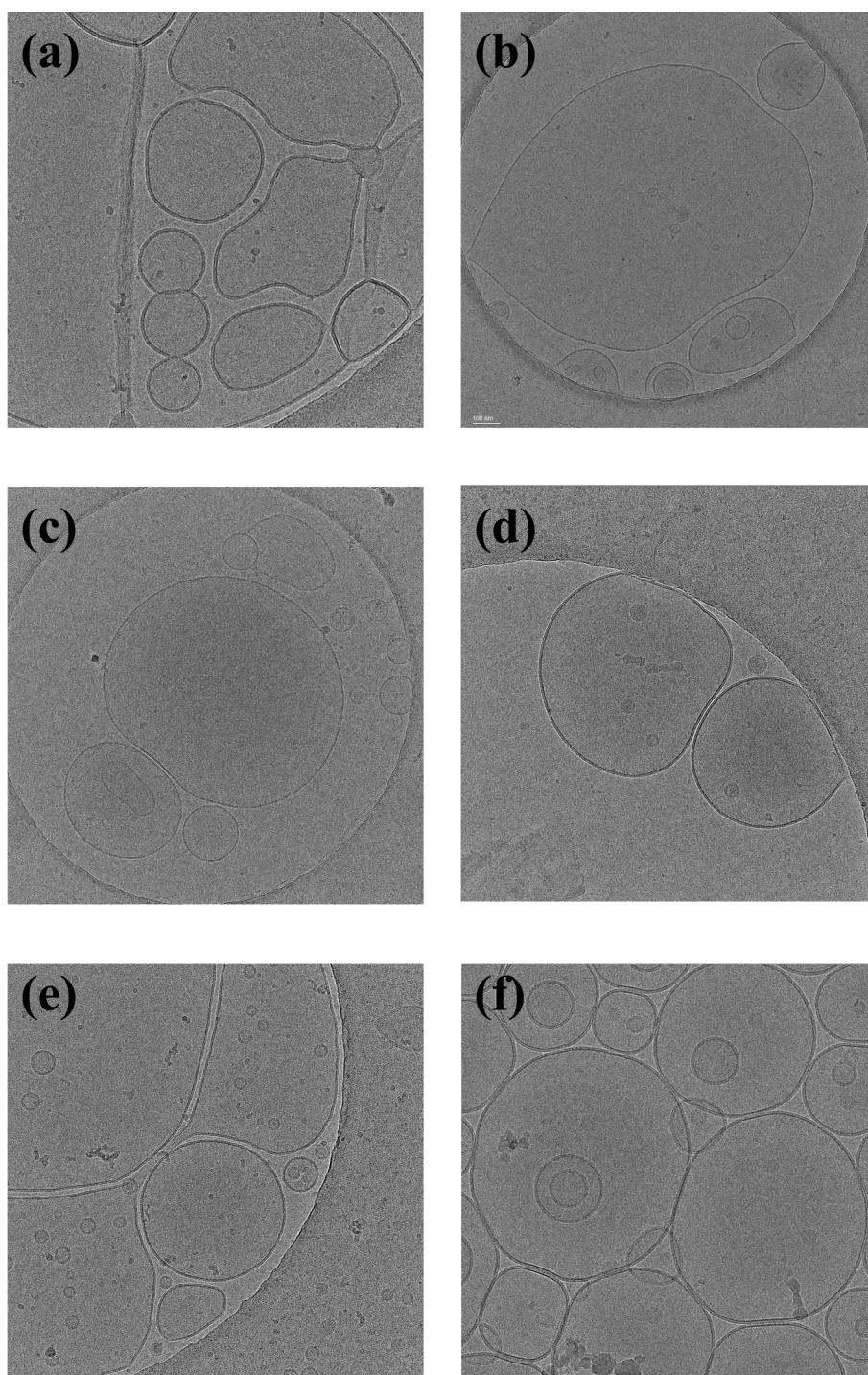


Fig. 5. Liposome loaded with NMN prepared by flask observed with the Cryo-TEM. (a) 600 rpm, (b) 650 rpm, (c) 700 rpm, (d) 750 rpm, (e) 1000 rpm, (f) 1500 rpm. (a)-(f) 47000x.

lamellar vesicles still dominate.

Gonzalez et al. [44] compared the synthesis of high solids acrylic pressure-sensitive adhesives using emulsion polymerization in both TCR and a continuous stirred tank reactor (CSTR). The results showed that TCR achieved higher conversion rates and narrower particle size distributions than CSTR. Additionally, the study demonstrated that TCR is suitable for synthesizing flocculants via inverse microemulsion polymerization. [44] Wei et al. [53] found that by varying the Taylor number, steady-state monomer conversion and particle number could be controlled within two limits corresponding to ideal plug flow reactor

(PFR) and CSTR simply by changing the rotational speed of the inner cylinder. Another significant characteristic of TCR is its ability to maintain very stable long-term operation without shear-induced coagulation or polymer deposition on the inner and outer cylinder surfaces. [53] The utilization of a TCR offers several advantages over conventional methods for liposome production. Controlling the speed of the inner cylinder allows for both the PFR to achieve high yields and the CSTR to provide uniform synthesis.

In the study by Varona et al. [54], liposomes were created using the thin-film hydration method and the particles from gas-saturated

solutions (PGSS) method. The particle sizes obtained from the thin-film method ranged from 0.4 to 1.3 μm , while the PGSS process yielded particles in the range of 1.4 to 24.8 μm . Additionally, liposomes produced by both methods exhibited multivesicular and multilamellar structures. Bridson et al. [55] developed liposomes using a modified compressed carbon dioxide solvent system. Their investigation focused on two methods for producing liposomes using compressed carbon dioxide in either its liquid or supercritical state as potential alternatives to conventional techniques. The first technique employed modified compressed carbon dioxide as a solvent system, examining how changes in pressure, temperature, apparatus geometry, and solvent flow rate affected the size distributions of the formulations. Generally, liposomes in the nano-size range with an average diameter of 200 nm could be produced, although some micro-sized vesicles were also present. The second method utilized compressed carbon dioxide as an antisolvent to promote uniform precipitation of phospholipids from concentrated ethanolic solutions, resulting in finely divided solvent-free phospholipid powders of saturated lipids that were subsequently hydrated to produce liposomes with mean volume diameters of around 5 μm . A clinical trial conducted by Davis et al. [56] demonstrated that liposomal encapsulation resulted in an almost 50 % increase in circulating vitamin C levels. In a separate study, Telange et al. [57] showed that liposomes significantly enhanced the delivery of apigenin over a 24-h period compared to a pure suspension. Similarly, Ye et al. [58] utilized liposomes to deliver NADH in mouse models, achieving a 25 % increase in the cellular NAD^+/NADH ratio, thereby improving therapeutic efficacy in sepsis. These findings, along with numerous other studies [59,60], underscore the remarkable versatility and efficiency of liposomes as drug carriers. This versatility arises from their capacity to encapsulate both water-soluble and fat-soluble molecules, which enhances stability and bioavailability. [61] Such characteristics position liposomes as a pivotal component in modern drug delivery systems, facilitating improved therapeutic outcomes. [62] In a recent study, Yu et al. [63] prepared a food-grade Pickering emulsion stabilizer by physically adsorbing the cationic surfactant ethyl lauroyl arginate onto the surface of nanomontmorillonite. This process resulted in an emulsion with an average droplet size of 1.0 μm . [63] Yu et al. [64] demonstrated that emulsified solid particles at the oil–water interface are crucial for stabilizing Pickering emulsions by preventing oil droplet coalescence. They used cellulose nanofibers (CNFs) as a low-cost stabilizer and dissolved NMN in sunflower oil, which enhanced emulsion stability. [64] To facilitate the oral delivery of NMN, researchers investigated the use of food proteins, specifically soy protein isolate (SPI) and whey protein isolate (WPI). Li et al. [65] explored the potential of SPI and NMN complexes as pH-responsive stabilizers for Pickering emulsions. [65] Additionally, Li et al. [66] demonstrated that the complexation of NMN with WPI via hydrogen bonds strengthens fish oil Pickering emulsions, creating a robust network that is resilient to varying pH conditions. [66]

At higher rpm (1000 rpm and 1500 rpm), the liposomes created using the TCR showed improved characteristics compared to previous studies. They exhibited smaller sizes and maintained particle separation, which enhanced their stability. Furthermore, while traditional methods require various materials such as compressed gas solvents and antisolvents and involve complex processes for liposome synthesis, utilizing TCR allows for the synthesis of high-quality liposomes in a single step, making it a valuable approach. This efficiency not only simplifies the production process but also contributes to the overall effectiveness of drug delivery systems. Overall, the advancements in liposome production techniques, particularly through TCR, highlight the potential for improved drug delivery applications, ensuring better stability and encapsulation efficiency, which are crucial for therapeutic effectiveness.

3.3. Zeta potential and size of NMN-loaded liposomes produced using TCR

Zeta potential is defined as the electrical potential at the slipping

plane of a colloidal nanoparticle, which is the boundary layer where the particle's surface charge influences the movement of surrounding ions. When a colloidal particle is dispersed in a liquid, it acquires a surface charge due to various factors, including ion adsorption, dissociation of surface groups, or the presence of surfactants. This surface charge attracts counter-ions of opposite charge from the surrounding medium, forming a tightly bound layer known as the Stern layer. Beyond this layer lies a diffuse layer of counter-ions, which balances the remaining surface charge of the particle. The zeta potential is a crucial parameter because it reflects the balance between attractive and repulsive forces acting on the particles. A high absolute value of zeta potential (either positive or negative) indicates strong electrostatic repulsion between particles, which helps to prevent aggregation. Conversely, a low zeta potential suggests that the repulsive forces are weak, leading to a higher likelihood of particle agglomeration. [67] The equipment used for this analysis was the conversion of measured electrophoretic mobility into zeta potential according to the Smoluchowski equation. [68]

Fig. 6 shows zeta potential data according to the change in rpm. The presence of phosphatidylcholine in the lipid bilayer of liposomes contributes to their negative charge. At higher rpm, phosphatidylcholine disperses more effectively, leading to the formation of more liposomes and resulting in a stronger negative charge. [69] As shown in the CFD analysis, at 600 rpm and 650 rpm where Taylor-Couette flow is not properly formed, the average zeta potential values were -17.91 ± 6.13 mV and -18.72 ± 7.73 mV, respectively. Additionally, the zeta potential values at 600 rpm and 650 rpm, where stable Taylor-Couette flow was not established, showed no statistically significant difference. ($p > 0.05$) At 700 rpm, where Taylor-Couette flow begins to be observed in the CFD simulation, the average zeta potential was -28.06 ± 9.03 mV. This value indicates that there was a significant difference in the zeta potential values before the formation of Taylor-Couette flow ($p < 0.05$). These values show negative charges on the particle surfaces, but the absolute value of the charge is lower than the critical value of -30 mV necessary for stable emulsification, suggesting inadequate stabilization. From 750 rpm to 1000 rpm and 1500 rpm, where Taylor-Couette flow is fully developed according to the CFD results, the average zeta potential values were -38.66 ± 7.41 mV, -38.10 ± 9.08 mV, and -40.13 ± 10.9 mV, respectively (Table 2). These values showed a statistically significant difference compared to the values at 700 rpm, where Taylor-Couette flow was not fully developed ($p < 0.05$). While there was no statistically significant difference in zeta potential between 750 rpm and 1000 rpm ($p > 0.05$), there was a significant difference when compared to the zeta potential at 1500 rpm ($p < 0.05$). These findings indicate that inter-particle repulsion forces are acting, ensuring stable emulsification. The particle size of the prepared liposome was also figured out according to the change in rpm (Fig. 7). Optimal particle size ranges from 120 nm to 160 nm; particles too small may not encapsulate NMN effectively, while those too large may not penetrate the skin's epidermal layer. As seen in the CFD analysis, increasing rpm results in higher fluid shear stress. Fig. 6 illustrates that with increasing rpm, more negative charge is applied, leading to stronger repulsion between particles and thus enhanced stability.

Holsaeter et al. [70] conducted an analysis of zeta potential after preparing liposomes with different chain lengths of polyethylene glycols (PEGs). The results indicated that PEG750, which had a higher absolute value of zeta potential, showed significantly less change in liposome size after 4 weeks compared to PEG2000. Similarly, the change in Docetaxel entrapment was also less with PEG750, demonstrating greater stability. [70] Therefore, a higher absolute value of zeta potential indicates that the liposomes formed are stable and less likely to aggregate.

Furthermore, Fig. 7 and Fig. 8(a) show that increasing rpm correlates with decreasing average particle size and dispersion (standard deviation), resulting in the production of more consistent and suitable liposome particles. The size distribution was analyzed according to the Stokes-Einstein formula. [71,72] Uniformity in liposome size is also crucial. At 600 rpm, the liposome size measures 185 ± 109 nm, showing

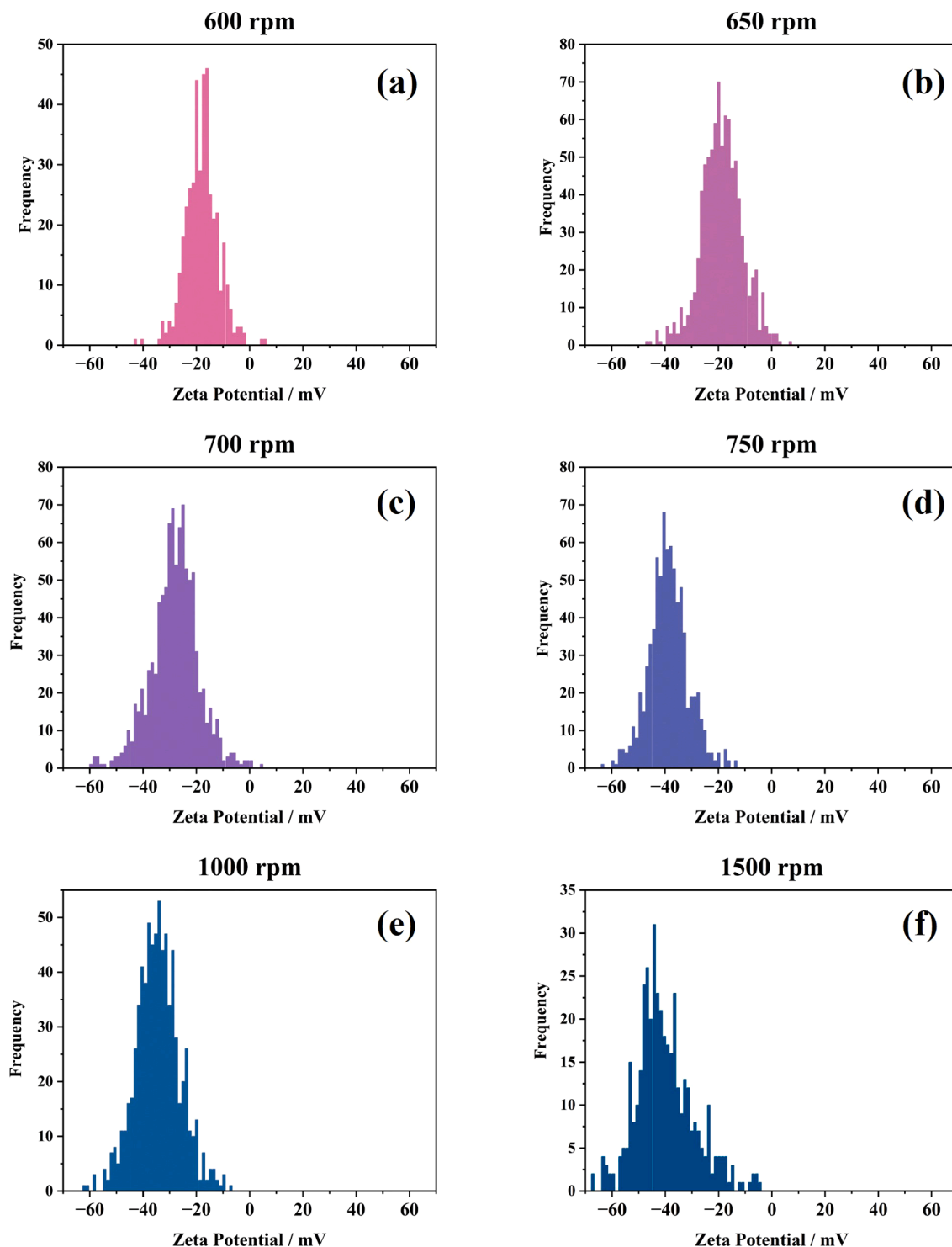


Fig. 6. Zeta potential according to rpm change. (a) 600 rpm, (b) 650 rpm, (c) 700 rpm, (d) 750 rpm, (e) 1000 rpm, (f) 1500 rpm.

Table 2

Zeta potential according to rpm change. a-e: different letters indicate significant differences ($p < 0.05$).

rpm	600	650	700	750	1000	1500
Zeta potential(mV)	-17.91 ± 6.13^a	-18.72 ± 7.73^a	-28.06 ± 9.03^b	-38.66 ± 7.41^c	-38.10 ± 9.08^c	-40.13 ± 10.9^c

large and non-uniform particles due to the absence of Taylor-Couette flow formation inside the TCR. This is clear from the CFD analysis. At 650 rpm, the liposome size decreases to 162 ± 84.7 nm, showing a

decrease in both size and standard deviation with increasing rpm. This indicates a statistically significant difference compared to the values at 600 rpm. Although the Taylor-Couette flow is formed at 700 rpm in the

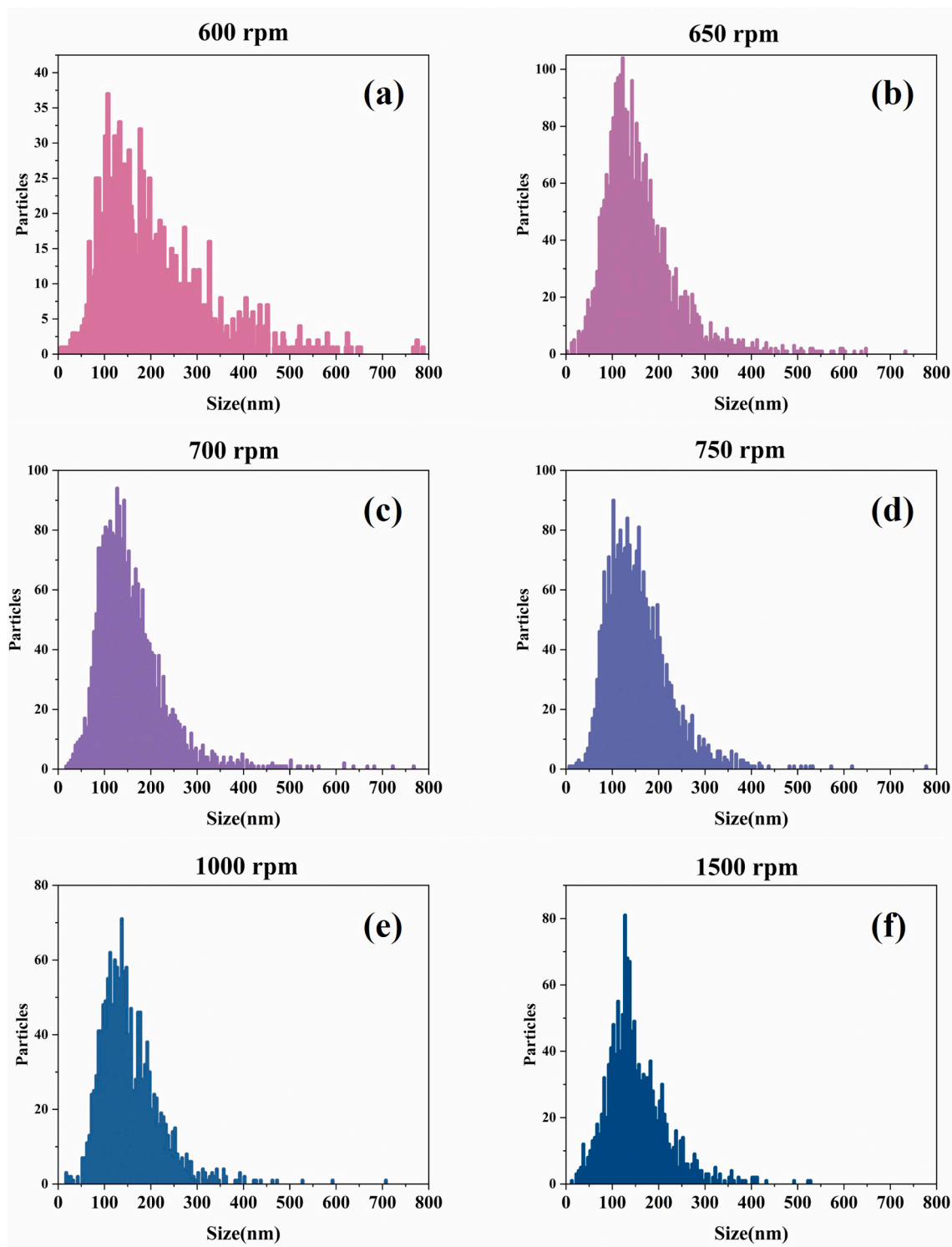


Fig. 7. Size of particles according to rpm change. (a) 600 rpm, (b) 650 rpm, (c) 700 rpm, (d) 750 rpm, (e) 1000 rpm, (f) 1500 rpm.

CFD analysis, it has not fully developed. As a result, the differences in fluid shear stress are not significant, ($p > 0.05$) leading to statistically insignificant differences between the values at 650 rpm and 700 rpm, which are 158 ± 77.1 nm ($p < 0.05$). From the value at 750 rpm, where Taylor-Couette flow is fully developed, a statistically significant difference is observed at 157 ± 70.1 nm, ($p < 0.05$) showing a consistent trend of decreasing particle size and standard deviation, resulting in smaller and more uniform particles. Increasing the rotational speed to 1000 rpm significantly reduces the mean size and standard deviation of

the particles to 145 ± 57.2 nm, showing a more pronounced decrease in size. Similarly, at 1500 rpm, the Taylor-Couette flow generates strong fluid shear stress, resulting in the production of small and uniform liposomes measuring 125 ± 47.6 nm. From 750 rpm to 1500 rpm, where stable Taylor-Couette flow is formed, it can be observed that the size of the liposomes decreases linearly ($y = -0.0415x + 187$, $R^2 = 0.999$). The values of the liposomes produced at 750 rpm, 1000 rpm, and 1500 rpm all show statistically significant differences ($p < 0.05$). In comparison to previous studies shown in Table 3, the results of this research indicate

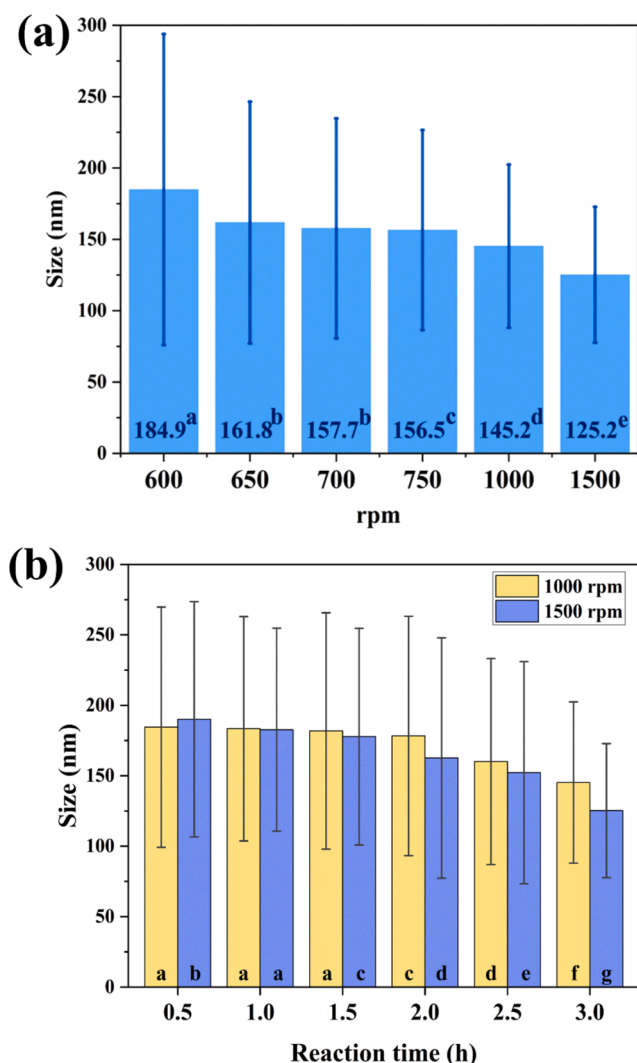


Fig. 8. Average size of liposomes in a Taylor-Couette reactor. (a) Effect of rpm. a-e: different letters indicate significant differences ($p < 0.05$), (b) effect of operation time. a-g: different letters indicate significant differences ($p < 0.05$).

that the liposomes have smaller sizes and lower zeta potential values. [73,74]

The results of the sampling at 1000 rpm and 1500 rpm are presented in Fig. 8(b). At 1000 rpm, a complete liposome was not formed until 2.0 h, remaining around 180 nm. However, by 2.5 h, the size significantly decreased to 160.1 ± 73.1 nm, which is small enough to potentially reach the dermal layer. At 1500 rpm, a complete liposome was also not formed until 1.5 h, maintaining a size of around 180 nm. By 2.0 h, the size is reduced to 162.6 ± 85.3 nm, allowing passage through the stratum corneum and epidermis. Statistical analysis reveals that there are no significant differences between the samples reacted for 0.5 h, 1.0 h, and 1.5 h at 1000 rpm and the sample reacted for 1.0 h at 1500 rpm ($p > 0.05$). However, a reaction time of 2 h at 1000 rpm results in a statistically significant difference compared to the previous values ($p < 0.05$). This can be considered equivalent to the sample reacted for 1.5 h at 1500 rpm based on statistical analysis ($p < 0.05$). Similarly, a reaction time of 2.5 h at 1000 rpm shows statistical similarity to the sample reacted for 2.0 h at 1500 rpm ($p < 0.05$). This trend indicates that by increasing the rpm, liposomes with similar characteristics can be synthesized in a shorter time. At 2.5 h, the size further decreased to 152.2 ± 78.8 nm. This indicates the changes in liposome size with respect to operation time. Effective liposomes began to form at 2.5 h for 1000 rpm and at 2.0 h for 1500 rpm. This suggests that increasing the rpm to

Table 3

Comparison of size and zeta potential of liposomes formed by different methods.

Drug	Methods	Size (nm)	Zeta potential (mV)	Reference
Artesunate	Thin film hydration	201.92	-23.97	(Wang et al., 2022) [73]
Artesunate	Ethanol injection	103.10	-18.93	(Wang et al., 2022) [73]
Artesunate	Modified thin film hydration	176.32 ± 2.02	-25.79 ± 0.22	(Wang et al., 2022) [73]
Lavandin essential oil	Thin film hydration	400–1300	–	(Varona et al., 2011) [54]
Lavandin essential oil	Gas-saturated solutions (PGSS)	1400–24800	–	(Varona et al., 2011) [54]
Cationic gene	NanoAssemblr	105 ± 19	35.8 ± 5	(Elsana et al., 2019) [74]
Cationic gene	NanoAssemblr (lipoplex)	149 ± 20	34.1 ± 3	(Elsana et al., 2019) [74]
Cationic gene	Rotary evaporator thin film hydration	294 ± 29	55.0 ± 2	(Elsana et al., 2019) [74]
Cationic gene	Rotary evaporator thin film hydration (lipoplex)	204 ± 23	50.6 ± 4	(Elsana et al., 2019) [74]
NMN	Taylor-Coutte reactor (1000 rpm)	145 ± 57.2	-38.10 ± 9.08	This study
NMN	Taylor-Coutte reactor (1500 rpm)	125 ± 47.6	-40.13 ± 10.9	This study

enhance shear stress may lead to a shorter reaction time.

This analysis demonstrates a clear trend where increasing rpm correlates with a reduction in both the average size and dispersion of liposome particles, leading to more uniform and consistent formulations. In the study by Hwang et al. [46], the size of $\text{Cu}(\text{OH})_2\text{-SiO}_2$ composite particles decreased from 13 μm at 300 rpm to 10 μm at 600 rpm, and further to 6 μm at 1000 rpm ($y = -0.01x + 16$, $R^2 = 1$). Through this study and Hwang's research, it can be observed that the particle size changes linearly with rpm variations within the stable Taylor-Couette flow regime. [46] At lower speeds, liposomes tend to be larger and more variable due to inadequate fluid dynamics, particularly the lack of Taylor-Couette flow. As the rpm increases, there is a significant decrease in particle size and standard deviation, reflecting improved uniformity. At higher rotational speeds, the strong fluid shear stress generated enhances the mixing of the oil and aqueous phases, leading to a faster elimination of the interface and more stable dispersion, resulting in the formation of smaller and more uniform liposomes. The findings indicate that effective liposome formation is also time-dependent, with optimal sizes reached after specific durations at elevated speeds. This highlights the importance of both rotational speed and operation time in producing liposomes that can effectively penetrate deeper skin layers. Overall, optimizing these parameters is crucial for the development of effective liposome-based delivery systems.

3.4. Stability of NMN-loaded liposomes

NMN is known to be unstable in aqueous solutions, especially at room temperature, where it can decompose into NAM. [19] This decomposition can significantly affect the efficacy of NMN as a supplement, particularly in formulations intended for long-term storage and use. Therefore, after storing NMN aqueous solution and NMN liposome solution made with TCR at 25°C for 180 days, the extent of degradation was observed. The degradation analysis was performed using HPLC,

with measurements conducted at intervals of 30, 90, and 180 days.

As detailed in Table 4, NMN sample stored in an aqueous solution at room temperature (25 °C) for 30 days exhibited a remaining NMN of only 0.321 ± 0.0651 %. This finding indicates that the NMN in the aqueous solution undergoes significant degradation, effectively rendering it nearly completely degraded. Such a high level of degradation underscores the challenges associated with storing NMN in liquid form, particularly for extended periods. In contrast, the NMN liposome samples created using TCR demonstrated remarkable stability. The liposome formulation acts as a protective barrier, encapsulating the NMN and shielding it from environmental factors that contribute to its degradation.

The findings showed that the sample prepared at 600 rpm maintained 84.9 ± 0.372 % of its NMN content after 30 days, indicating that a significant majority of the NMN remained intact. However, over time, the NMN content decreased to 68.3 ± 1.96 % at 90 days and 55.8 ± 1.37 % at 180 days, indicating degradation due to structural instability of the liposomes. These changes in NMN concentration are statistically significant. The liposomes synthesized when Taylor-Couette flow was not fully established exhibited structural instability due to insufficient shear stress. In contrast, the sample at 750 rpm exhibited a higher stability of 85.9 ± 0.554 % after 30 days. After 90 days, some instability in the synthesized liposomes led to a reduction in NMN content to 80.9 ± 1.32 %. However, after 180 days, 80.2 ± 0.524 % of NMN remained, indicating that the majority of the stably synthesized liposomes effectively stabilized the NMN. Statistical analysis suggests that there is no significant difference between the remaining NMN amounts at 90 days and 180 days. The samples prepared at higher rotational speeds also demonstrate impressive stability. The 1000 rpm sample retained 90.3 ± 0.520 % of its NMN content after 30 days. After 90 days, this decreased to 87.6 ± 0.12 %, indicating that approximately 2.7 % of NMN was lost due to the breakdown of a small number of unstable liposomes. At 180 days, the remaining NMN content was 87.0 ± 0.662 %, and statistical analysis indicated no significant difference between the remaining NMN amounts at 90 days and 180 days. Meanwhile, the 1500 rpm sample showed a higher retention rate of 91.6 ± 0.323 % after 30 days. Due to the highly stable synthesis of liposomes at this rotational speed, the NMN content remained at 89.8 ± 0.233 % after 90 days and 89.4 ± 1.44 % after 180 days. All three values are statistically similar, indicating that NMN encapsulated in liposomes synthesized at 1500 rpm remains highly stable when stored at room temperature for six months. This suggests significant potential for long-term storage. By transforming NMN, which decomposes rapidly in aqueous solution, into liposomal formulations, this study contributes greatly to the advancement of NMN delivery systems. These results highlight the effectiveness of the liposomal encapsulation method in preserving NMN compared to the aqueous solution. NMN is a zwitterion that contains a positively charged amine group and a negatively charged carboxyl group. It is electrically stabilized by being surrounded by negatively charged phosphatidylcholine, which helps prevent decomposition in aqueous solutions. As the number of liposomes increases, NMN becomes more stabilized. This indicates that in solutions with a higher turbidity due to a greater number of liposomes, NMN remains more stable. [75,76] These findings underscore the importance of formulation strategies in preserving the integrity of NMN. Since NMN tends to decompose in

aqueous solution, NMN not encapsulated in liposomes will degrade and become undetectable by HPLC (Table 4). Therefore, we can indirectly determine the encapsulation efficiency by measuring the amount of NMN remaining in the liposome solution after 30, 90, and 180 days at room temperature.

To assess the stability of liposomes under various temperature conditions, samples were stored at 25 °C (room temperature), 40 °C, and 60 °C in an oven for 30 days. HPLC was performed to evaluate the stability of the liposomes at 15 and 30 days, and the results are presented in Table 5. After 15 days of the experiment, the NMN content in the aqueous solution at 25 °C was found to be 4.93 ± 0.512 %, indicating rapid decomposition of NMN. At 40 °C, only 1.37 ± 0.08 % remained, and at 60 °C, NMN was completely degraded. In contrast, the NMN content in liposomes prepared at 1500 rpm and stored at 25 °C for 15 days remained at 99.0 ± 0.617 %, demonstrating the effectiveness of liposomal encapsulation. The amount of NMN remaining in liposomal formulations varied depending on the stability of the liposomes. In the case of liposomes prepared at 600 rpm, where Taylor-Couette flow was not established, 85.4 ± 1.60 % of NMN remained after 15 days at 25 °C, and 84.9 ± 0.372 % after 30 days, indicating a considerable amount of NMN retention. However, at 40 °C, the structural instability of the liposomes led to phase separation, resulting in a reduction of the remaining NMN by approximately half. This trend was consistent at both 15 and 30 days. At 700 rpm, as the temperature increased, the structural stability of the liposomes decreased, leading to a reduction in the amount of NMN remaining. However, the stability at 600 rpm at 40 °C was statistically different from the data obtained at room temperature. The remaining NMN amounts at 15 and 30 days were 66.3 ± 3.14 % and 64.8 ± 4.54 %, respectively, which represents an approximately 45 % improvement compared to the 600 rpm samples. This trend was also observed at 700 and 750 rpm. Notably, at 750 rpm, the NMN retentions at 40 °C after 15 and 30 days were 78.6 ± 3.66 % and 76.2 ± 3.55 %, respectively, indicating a statistically significant difference compared to the 700 rpm samples. This suggests that liposomes exhibiting stability at both room temperature and elevated temperatures have begun to be produced. From 1000 rpm onwards, it was observed that stable liposomes were produced at 40 °C. The NMN content remaining at 25 °C after 30 days was 90.3 ± 0.520 %, while at 40 °C, it was 85.7 ± 5.56 %. These values showed no statistically significant difference, indicating similar stability at both temperatures. This trend was also observed at 1500 rpm. The NMN amounts remaining in liposome solutions stored at 25 °C for 30 days and those stored at 40 °C for 15 and 30 days were 91.6 ± 0.323 %, 93.9 ± 1.72 %, and 86.4 ± 5.88 %, respectively, with no statistically significant differences. However, at 60 °C, the structural stability of the liposomes deteriorated rapidly, leading to phase separation between the oil and aqueous phases. Consequently, the samples analyzed after 15 days at 60 °C showed that almost all NMN

Table 4

Long-term stability of NMN in aqueous solution and liposomal form after 30, 90, and 180 days. a-g: different letters indicate significant differences ($p < 0.05$).

Remaining NMN (%)	30 days	90 days	180 days
In aqueous solution	0.321 ± 0.0651^a	0.259 ± 0.520^a	0.213 ± 0.323^a
600 rpm	84.9 ± 0.372^b	68.3 ± 1.96^c	55.8 ± 1.37^d
750 rpm	85.9 ± 0.554^b	80.9 ± 1.32^e	80.2 ± 0.524^e
1000 rpm	90.3 ± 0.520^f	87.6 ± 0.121^g	87.0 ± 0.662^g
1500 rpm	91.6 ± 0.323^f	89.8 ± 0.233^f	89.4 ± 1.44^f

Table 5

Degradation of NMN in aqueous solution and liposomal form at various temperatures after 15 and 30 days. a-j: different letters indicate significant differences ($p < 0.05$).

Remaining NMN (%)	25 °C		40 °C		60 °C
	15 days	30 days	15 days	30 days	15 days
In aqueous solution	4.93	0.321	1.37	0.275	0
600 rpm	$\pm 0.512^a$	$\pm 0.0651^b$	$\pm 0.08^c$	$\pm 0.214^b$	
	85.4	84.9	45.6	42.8	1.59
	$\pm 1.60^d$	$\pm 0.372^d$	$\pm 5.78^e$	$\pm 8.94^e$	$\pm 0.290^c$
700 rpm	89.1	85.9	66.3	64.8	2.24
	$\pm 1.92^d$	$\pm 0.325^d$	$\pm 3.14^h$	$\pm 4.54^h$	$\pm 0.285^j$
750 rpm	91.3	85.9	78.6	76.2	2.46
	$\pm 2.07^d$	$\pm 0.554^d$	$\pm 3.66^i$	$\pm 3.55^i$	$\pm 0.173^j$
1000 rpm	94.5	90.3	91.6	85.7	3.36
	$\pm 0.667^e$	$\pm 0.520^g$	$\pm 0.415^g$	$\pm 5.56^g$	$\pm 0.367^j$
1500 rpm	99.0	91.6	93.9	86.4	5.34
	$\pm 0.617^f$	$\pm 0.323^g$	$\pm 1.72^g$	$\pm 5.88^g$	$\pm 0.351^a$

decomposed. After 30 days at 60°C, no NMN peak was detected in any sample, indicating that the liposomal structure, similar to the cell membrane, is disrupted at elevated temperatures. As research continues to explore the benefits of NMN, the development of stable and effective formulations will be crucial in maximizing its therapeutic potential.

3.5. Ex vivo test of NMN-loaded liposomes

The barrier known as the stratum corneum has a thickness of 10–20 μm . Below this layer, there is an avascular epidermis measuring 50–100 μm . Beneath that lies the dermis. Our goal is to determine whether NMN reaches the dermal layer, so we conducted experiments using 400 μm porcine skin membranes. [77] The experiment was conducted using Franz cell diffusion and quantified through HPLC. The structure of the Franz cell is illustrated in Fig. 9(a). We used piglet skin membranes, which closely resemble human skin, as the barrier in the experiment. [78] When comparing the absorption of liposome loaded with NMN with and without applying a driving force, applying a driving force directly to the skin can accelerate the rate at which the liquid penetrates a surface with cavities. When liposomes are applied to the skin and tapped, they can more easily pass through the tiny pores of the epidermis. This facilitates rapid and efficient absorption. [79]

As depicted in Fig. 9(b), the cumulative release percentages of the

results show that in the presence of a driving force, $96.8 \pm 1.76\%$ of the liposomes permeate the skin. Whereas without the driving force, only $70.7 \pm 7.61\%$ permeate the skin. The driving force was applied by tapping the membrane 30 times over a duration of 5 min. In the absence of a driving force, residual liposomes stay on the skin surface or within the epidermal layer. Therefore, by applying liposomes to the skin and tapping, the concentration gradient allows liposomes to penetrate more rapidly and extensively than they would intrinsically.

Sabbagh and Kim [27] prepared various polyvinyl alcohol (PVA)-based microneedle patches to develop a transdermal delivery system for NMN. The highest NMN release of 91.94 % in 18 h was obtained using a 9.5 kDa molecular weight PVA microneedles containing NMN and carboxymethyl cellulose. In this study, the NMN penetration percentage of $96.8 \pm 1.76\%$ within 12 h obtained using liposomes with the application of a driving force was higher than the highest release percentage obtained using PVA microneedles. Additionally, it was observed that NMN hydrolyzes to NAM when the drug concentration in the microneedle is high, suggesting that the drug concentration should be determined to reduce drug hydrolysis. [27] The use of liposomes increases stability and reduces the hydrolysis of NMN to NAM, representing a promising alternative to NMN microneedles.

Recent liposome research has focused on treating photodamage by liposomalizing high molecular weight hyaluronic acid to create a transdermal system. Liposomes were prepared using methods such as reverse evaporation, high-speed homogenization, and micro-jet techniques. A murine model of acute skin injury induced by laser ablation was established, demonstrating that the transdermal system exhibited significant anti-inflammatory and immunosuppressive properties, promoting skin proliferation and scar repair, thus highlighting its potential in accelerating skin wound healing. [80] In another study, a nano-formulated curcumin-fused lipogel was developed, which showed enhanced wound healing ability and sustained drug release. The promotion of angiogenesis was confirmed through an in-ovo CAM assay, and antimicrobial effects were validated using the Kirby-Bauer assay against *E. coli* and *Staphylococcus aureus*. [81] Additionally, liposomes were used to reduce nonsteroidal anti-inflammatory drug (NSAID)-related adverse effects. A chitosan-based hydrogel was developed using liposomes loaded with aceclofenac (ACE), adopting a central composite design to enhance synergistic anti-inflammatory efficacy and improve the dermal administration of ACE. [82]

Future research can focus on efficient production and delivery methods of liposomes. Increasing the viscosity to apply greater shear stress to the fluid can reduce the reaction time and allow for a continuous process. This will simplify the commercial liposome manufacturing process to a single step, contributing to the establishment of an efficient production system suitable for industrial applications. Additionally, research can be conducted to produce liposomes containing NMN in the form of microneedles to directly deliver NMN into the dermal layer. Microneedles are tiny, minimally invasive needles that can penetrate the outer layer of the skin without causing significant pain. They create microchannels that allow for the efficient delivery of therapeutic agents directly into the deeper skin layers, enhancing absorption and efficacy. This innovative delivery method holds great promise for improving the bioavailability of NMN and other active ingredients in cosmetic and pharmaceutical applications.

4. Conclusion

This study aimed to address the challenges associated with the bioavailability, stability, and delivery of NMN through the production of NMN-loaded liposomes using a TCR. Through a comprehensive investigation, several key findings and advancements were achieved. Firstly, the adoption of liposomes as a delivery system for NMN showed promise in overcoming stability and bioavailability issues. The TCR facilitated uniform mixing and dispersion of NMN within the lipid bilayers, resulting in stable liposomes. Furthermore, the prepared NMN-loaded

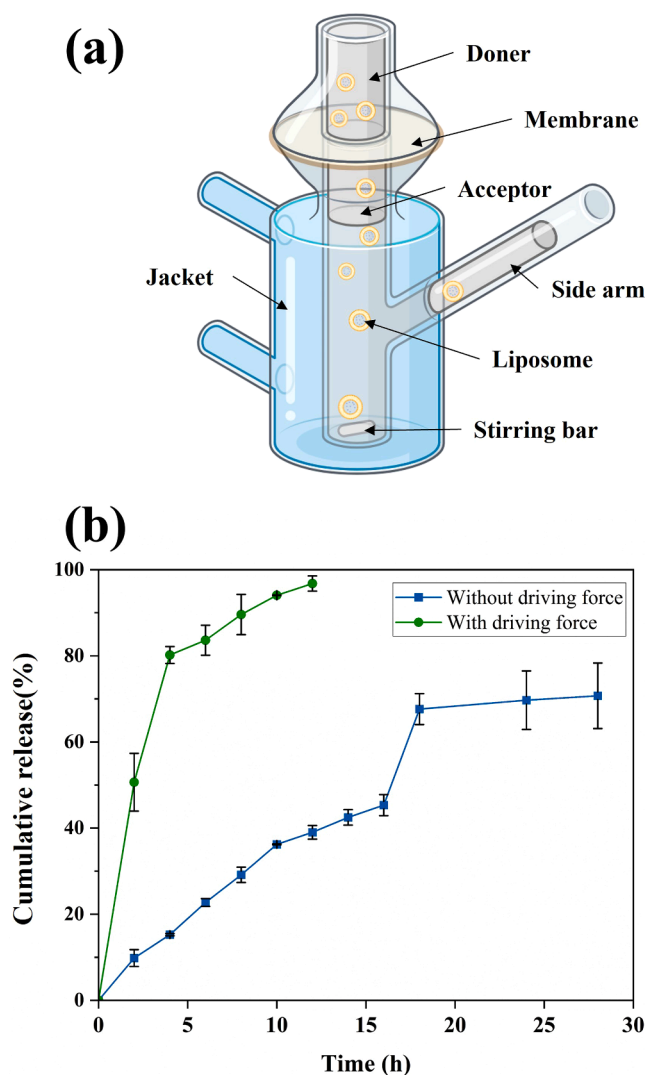


Fig. 9. (a) Structure of the Franz cell and (b) ex vivo pig skin permeability measured with Franz cell.

liposomes were characterized for their physicochemical properties and *ex vivo* pig skin penetration test. The results demonstrated the ability of TCR-produced liposomes to achieve stable emulsification and uniform particle size, compared to conventional methods. Stable liposomes were consistently formed at 750 rpm and above, as evidenced by CFD simulations, optical properties of the dispersion solution, zeta potential, and particle size analysis. Particularly, at 1000 rpm, liposomes showed a size of 145 ± 57.2 nm and an average zeta potential of -38.10 ± 9.08 mV, while at 1500 rpm, the size reduced to 125 ± 47.6 nm with an average zeta potential of -40.13 ± 10.9 mV. These dimensions and zeta potential values are sufficiently small and stable to penetrate the epidermal layer effectively, ensuring the stable delivery of NMN and preventing its degradation. Liposomes prepared at 1000 rpm and 1500 rpm showed stabilization effects for NMN, remaining at 87.0 ± 0.662 % and 89.4 ± 1.44 %, respectively, after 180 days at room temperature. The *ex vivo* testing using Franz cell diffusion further validated the efficacy of NMN-loaded liposomes in penetrating the skin barrier. The results showed a significant increase in penetration percentage when a driving force was applied, highlighting the potential of liposomes for targeted drug delivery to the skin. Without a driving force, the penetration percentage converges to 70.7 ± 7.61 % after 18 h. However, with the application of a driving force, the penetration percentage reaches 96.8 ± 1.76 % within 12 h, showing that mostly all liposomes penetrate the dermal layer. In summary, this study provides valuable insights into the production and characterization of NMN-loaded liposomes, paving the way for the development of novel delivery systems for NMN. By addressing key challenges in stability, bioavailability, and delivery, NMN-loaded liposomes prepared using a TCR offer promising prospects for biomedical applications, potentially advancing the therapeutic potential of NMN in various health conditions and contributing to the field of nanomedicine.

CRediT authorship contribution statement

Isu Kim: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Beom Soo Kim:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This research was supported by the Regional Innovation Strategy through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (MOE) (2021RIS-001).

Data availability

Data will be made available on request.

References

- [1] J. Yoshino, J.A. Baur, S.I. Imai, NAD⁺ Intermediates: The Biology and Therapeutic Potential of NMN and NR, *Cell Metab.* 27 (2018) 513–528.
- [2] J. Yoshino, K.F. Mills, M.J. Yoon, S. Imai, Nicotinamide mononucleotide, a key NAD⁺ intermediate, treats the pathophysiology of diet- and age-induced diabetes in mice, *Cell Metab.* 14 (2011) 528–536.
- [3] C. Canto, R.H. Houtkooper, E. Pirinen, D.Y. Youn, M.H. Oosterveer, Y. Cen, P. J. Fernandez-Marcos, H. Yamamoto, P.A. Andreux, P. Cettour-Rose, K. Gademann, C. Rinsch, K. Schoonjans, A.A. Sauve, J. Auwerx, The NAD⁺ precursor nicotinamide riboside enhances oxidative metabolism and protects against high-fat diet-induced obesity, *Cell Metab.* 15 (2012) 838–847.
- [4] A.J. Covarrubias, R. Perrone, A. Grozio, E. Verdin, NAD⁺ metabolism and its roles in cellular processes during ageing, *Nat. Rev. Mol. Cell Biol.* 22 (2021) 119–141.
- [5] M. Soma, S.K. Lalam, The role of nicotinamide mononucleotide (NMN) in anti-aging, longevity, and its potential for treating chronic conditions, *Mol. Biol. Rep.* 49 (2022) 9737–9748.
- [6] H. Zhang, D. Ryu, Y. Wu, K. Gariani, X. Wang, P. Luan, D. D'Amico, E.R. Ropelle, M.P. Lutolf, R. Aebersold, K. Schoonjans, K.J. Menzies, J. Auwerx, NAD⁺ repletion improves mitochondrial and stem cell function and enhances life span in mice, *Science* 352 (2016) 1436–1443.
- [7] Z. Huang, N. Li, S. Yu, W. Zhang, T. Zhang, J. Zhou, Systematic Engineering of *Escherichia coli* for Efficient Production of Nicotinamide Mononucleotide From Nicotinamide, *ACS Synth. Biol.* 11 (2022) 2979–2988.
- [8] M. Yoshino, J. Yoshino, B.D. Kayser, G.J. Patti, M.P. Franczyk, K.F. Mills, M. Sindelar, T. Pietka, B.W. Patterson, S.-I. Imai, S. Klein, Nicotinamide mononucleotide increases muscle insulin sensitivity in prediabetic women, *Science* 372 (2021) 1224–1229.
- [9] S.I. Imai, L. Guarente, It takes two to tango: NAD⁺ and sirtuins in aging/longevity control, *npj Aging Mech. Dis.* 2 (2016) 16017.
- [10] A. Maharjan, M. Singhvi, B.S. Kim, Biosynthesis of a Therapeutically Important Nicotinamide Mononucleotide through a Phosphoribosyl Pyrophosphate Synthetase 1 and 2 Engineered Strain of *Escherichia coli*, *ACS Synth. Biol.* 10 (2021) 3055–3065.
- [11] S.R. Kafle, A. Kushwaha, L. Goswami, A. Maharjan, B.S. Kim, A holistic approach for process intensification of nicotinamide mononucleotide production via high cell density cultivation under exponential feeding strategy, *Bioresour. Technol.* 390 (2023) 129911.
- [12] A. Maharjan, M. Singhvi, S.R. Kafle, B.S. Kim, Enhanced production of nicotinamide mononucleotide by high cell density culture of engineered *Escherichia coli*, *Process Biochem.* 131 (2023) 264–271.
- [13] S.R. Kafle, R.B. Cabulong, B.S. Kim, A novel pH-stat fed-batch strategy to boost the production of nicotinamide mononucleotide by high cell density fermentation, *Chem. Eng. Sci.* 296 (2024) 120240.
- [14] Naidu, R., Semple, K. T., Megharaj, M., Juhasz, A. L., Bolan, N. S., Gupta, S. K., Clothier, B. E., and Schulin, R. (2008) Chapter 3 Bioavailability: Definition, assessment and implications for risk assessment, In *Chemical Bioavailability in Terrestrial Environment*, pp 39–51.
- [15] P. Borel, D. Preveraud, C. Desmarchelier, Bioavailability of vitamin E in humans: an update, *Nutr. Rev.* 71 (2013) 319–331.
- [16] M.J. Mitchell, M.M. Billingsley, R.M. Haley, M.E. Wechsler, N.A. Peppas, R. Langer, Engineering precision nanoparticles for drug delivery, *Nat. Rev. Drug Discov.* 20 (2021) 101–124.
- [17] L. Liu, X. Su, W.J. Quinn 3rd, S. Hui, K. Krukenberg, D.W. Frederick, P. Redpath, L. Zhan, K. Chellappa, E. White, M. Migaud, T.J. Mitchison, J.A. Baur, J. D. Rabinowitz, Quantitative Analysis of NAD Synthesis-Breakdown Fluxes, *Cell Metab.* 27 (2018) 1067–1080.e5.
- [18] M. Sharma, Transdermal and Intravenous Nano Drug Delivery Systems, In *Applications of Targeted Nano Drugs and Delivery Systems* (2019) 499–550.
- [19] T. Tanaka, M. Tanaka, R. Hiramatsu, D. Shimamura, High-purity β nicotinamide mononucleotide (NMN) and method for producing same, 2023. US20230416291.
- [20] Y.P. Patil, S. Jadhav, Novel methods for liposome preparation, *Chem. Phys. Lipids* 177 (2014) 8–18.
- [21] C. Cheng, S. Peng, Z. Li, L. Zou, W. Liu, C. Liu, Improved bioavailability of curcumin in liposomes prepared using a pH-driven, organic solvent-free, easily scalable process, *RSC Adv.* 7 (2017) 25978–25986.
- [22] A.C. Paiva-Santos, A.L. Silva, C. Guerra, D. Peixoto, M. Pereira-Silva, M. Zeinali, F. Mascarenhas-Melo, R. Castro, F. Veiga, Ethosomes as Nanocarriers for the Development of Skin Delivery Formulations, *Pharm. Res.* 38 (2021) 947–970.
- [23] S.T. Wereley, R.M. Lueptow, Velocity field for Taylor-Couette flow with an axial flow, *Phys. Fluids* 11 (1999) 3637–3649.
- [24] M. Schrimpf, J. Esteban, H. Warmeling, T. Färber, A. Behr, A.J. Vorholt, Taylor-Couette reactor: Principles, design, and applications, *AIChE J* 67 (2021) e17228.
- [25] P. Garcia-Manrique, N.D. Machado, M.A. Fernandez, M.C. Blanco-Lopez, M. Matos, G. Gutierrez, Effect of drug molecular weight onniosomes size and encapsulation efficiency, *Colloids Surf. B Biointerfaces* 186 (2020) 110711.
- [26] D.S. Tupuna, K. Paese, S.S. Guterres, A. Jablonski, S.H. Flores, A. Rios, d. O., Encapsulation efficiency and thermal stability of norbixin microencapsulated by spray-drying using different combinations of wall materials, *Ind. Crop. Prod.* 111 (2018) 846–855.
- [27] F. Sabbagh, B.S. Kim, Ex Vivo Transdermal Delivery of Nicotinamide Mononucleotide Using Polyvinyl Alcohol Microneedles, *Polymers* 15 (2023) 2031.
- [28] F. Otto, P. van Hoogevest, F. Syrowatka, V. Hehl, R.H.H. Neubert, Assessment of the applicability of HLB values for natural phospholipid emulsifiers for preparation of stable emulsions, *Pharmazie* 75 (2020) 365–370.
- [29] S. Peng, L. Zou, W. Liu, C. Liu, D.J. McClements, Fabrication and Characterization of Curcumin-Loaded Liposomes Formed from Sunflower Lecithin: Impact of Composition and Environmental Stress, *J. Agric. Food Chem.* 66 (2018) 12421–12430.
- [30] D. Clause, I. Pezron, L. Komunjer, Stability of W/O and W/O/W emulsions as a result of partial solidification, *Colloids Surf A Physicochem Eng Asp* 152 (1999) 23–29.
- [31] U. Hasanah, S. Warnasih, Synthesis and characterization of medium- chain triglyceride (MCT) from virgin coconut oil (VCO), *AIP Conference Proceedings* 2243 (2020) 363–372.
- [32] J.M. Morais, O.D.H. Santos, J.R.L. Nunes, C.F. Zanatta, P.A. Rocha-Filho, W/O/W Multiple Emulsions Obtained by One-Step Emulsification Method and Evaluation of the Involved Variables, *J. Dispers. Sci. Technol.* 29 (2008) 63–69.

- [33] S.F. Ng, J.J. Rouse, F.D. Sanderson, V. Meidan, G.M. Eccleston, Validation of a static Franz diffusion cell system for in vitro permeation studies, *AAPS PharmSciTech* 11 (2010) 1432–1441.
- [34] I.R. Silva, F.A. Lima, E.C.O. Reis, L.A.M. Ferreira, G.A.C. Goulart, Stepwise Protocols for Preparation and Use of Porcine Ear Skin for in Vitro Skin Permeation Studies Using Franz Diffusion Cells, *Curr Protoc* 2 (2022) e391.
- [35] V.N. Mehta, S.S. Chettiar, J.R. Bhamore, S.K. Kailasa, R.M. Patel, Green Synthetic Approach for Synthesis of Fluorescent Carbon Dots for Lisinopril Drug Delivery System and their Confirmations in the Cells, *J. Fluoresc.* 27 (2017) 111–124.
- [36] D.G. Altman, J.M. Bland, How to obtain the confidence interval from a P value, *BMJ* 343 (2011) d2090–d.
- [37] D.G. Altman, J.M. Bland, How to obtain the P value from a confidence interval, *BMJ* 343 (2011) d2304.
- [38] M. Rosenfeld, D. Kwak, M. Vinokur, A fractional step solution method for the unsteady incompressible navier-stokes equations in generalized coordinate systems, *J. Comput. Phys.* 94 (1991) 102–137.
- [39] J.-Y. Hwang, K.-S. Yang, Numerical study of Taylor-Couette flow with an axial flow, *Comput. Fluids* 33 (2004) 97–118.
- [40] M. Bilson, K. Bremhorst, Direct numerical simulation of turbulent Taylor-Couette flow, *Journal of Fluid Mechanics* 579 (2007) 227–270.
- [41] S. Grossmann, D. Lohse, C. Sun, High-Reynolds Number Taylor-Couette Turbulence, *Annu. Rev. Fluid Mech.* 48 (2016) 53–80.
- [42] J.S. Kim, D.H. Kim, B. Gu, D.Y. Kim, D.R. Yang, Simulation of Taylor-Couette reactor for particle classification using CFD, *J. Cryst. Growth* 373 (2013) 106–110.
- [43] S. Dong, Direct numerical simulation of turbulent Taylor-Couette flow, *J. Fluid Mech.* 587 (2007) 373–393.
- [44] G. González, E. Colmenar, G. Diaconu, F. Alarcia, M. Manea, M. Paulis, M. J. Barandiaran, J.R. Leiza, J.C. de la Cal, J.M. Asua, Production of Widely Different Dispersed Polymers in a Continuous Taylor-Couette Reactor, *Macromol. React. Eng.* 3 (2009) 233–240.
- [45] G. Li, X. Yang, H. Ye, CFD simulation of shear flow and mixing in a Taylor-Couette reactor with variable cross-section inner cylinders, *Powder Technol.* 280 (2015) 53–66.
- [46] Hwang, D., Lee, M., Chang, J., Hwang, Y., Lee, U., and Hong, D. (2016) A preparation method of composite particles using Taylor reactor, KR-20160028082-A.
- [47] K. Kataoka, H. Doi, T. Kongo, M. Futagawa, Ideal Plug-Flow Properties of Taylor Vortex Flow, *J. Chem. Eng. Jpn.* 8 (1975) 472–476.
- [48] M.F. Aljishi, A.-C. Ruo, J.H. Park, B. Nasser, W.-S. Kim, Y.L. Joo, Effect of flow structure at the onset of instability on barium sulfate precipitation in Taylor-Couette crystallizers, *J. Cryst. Growth* 373 (2013) 20–31.
- [49] O.S. Alade, M. Mahmoud, D.A. Al Shehri, A.S. Sultan, Rapid Determination of Emulsion Stability Using Turbidity Measurement Incorporating Artificial Neural Network (ANN): Experimental Validation Using Video/Optical Microscopy and Kinetic Modeling, *ACS Omega* 6 (2021) 5910–5920.
- [50] M. Almgren, K. Edwards, G. Karlsson, Cryo transmission electron microscopy of liposomes and related structures, *Colloids Surf A Physicochem Eng Asp* 174 (2000) 3–21.
- [51] N. Mignet, J. Seguin, M. Ramos Romano, L. Brulle, Y.S. Touil, D. Scherman, M. Bessodes, G.G. Chabot, Development of a liposomal formulation of the natural flavonoid fisetin, *Int. J. Pharm.* 423 (2012) 69–76.
- [52] R.L. Shew, D.W. Deamer, A novel method for encapsulation of macromolecules in liposomes, *Biochimica et Biophysica Acta (BBA) Biomembranes* 816 (1985) 1–8.
- [53] X. Wei, H. Takahashi, S. Sato, M. Nomura, Continuous emulsion polymerization of styrene in a single Couette-Taylor vortex flow reactor, *J. Appl. Polym. Sci.* 80 (2001) 1931–1942.
- [54] S. Varona, Á. Martín, M.J. Cocero, Liposomal Incorporation of Lavandin Essential Oil by a Thin-Film Hydration Method and by Particles from Gas-Saturated Solutions, *Ind. Eng. Chem. Res.* 50 (2011) 2088–2097.
- [55] R.H. Bridson, R.C. Santos, B. Al-Duri, S.M. McAllister, J. Robertson, H.O. Alpar, The preparation of liposomes using compressed carbon dioxide: strategies, important considerations and comparison with conventional techniques, *J. Pharm. Pharmacol.* 58 (2006) 775–785.
- [56] J.L. Davis, H.L. Paris, J.W. Beals, S.E. Binns, G.R. Giordano, R.L. Scalzo, M. M. Schweder, E. Blair, C. Bell, Liposomal-encapsulated Ascorbic Acid: Influence on Vitamin C Bioavailability and Capacity to Protect Against Ischemia-Reperfusion Injury, *Nutr. Metab. Insights* 9 (2016) 25–30.
- [57] D.R. Telange, A.T. Patil, A.M. Pethe, H. Fegade, S. Anand, V.S. Dave, Formulation and characterization of an apigenin-phospholipid phytosome (APLC) for improved solubility, in vivo bioavailability, and antioxidant potential, *Eur. J. Pharm. Sci.* 108 (2017) 36–49.
- [58] M. Ye, Y. Zhao, Y. Wang, R. Xie, Y. Tong, J.D. Sauer, S. Gong, NAD(H)-loaded nanoparticles for efficient sepsis therapy via modulating immune and vascular homeostasis, *Nat. Nanotechnol.* 17 (2022) 880–890.
- [59] H. Nsairat, W. Alshair, F. Odeh, E. Esawi, D. Khater, A.A. Bawab, M. El-Tanani, A. Awidi, M.S. Mubarak, Recent advances in using liposomes for delivery of nucleic acid-based therapeutics, *OpenNano* 11 (2023) 100132.
- [60] K.B. Khot, G. Gopan, A. Bandiwadekar, J. Jose, Current advancements related to phytobioactive compounds based liposomal delivery for neurodegenerative diseases, *Ageing Res. Rev.* 83 (2023) 101806.
- [61] N. Maurer, D.B. Fenske, P.R. Cullis, Developments in liposomal drug delivery systems, *Expert Opin. Biol. Ther.* 1 (2001) 923–947.
- [62] C. Benjamin, R. Crews, Nicotinamide Mononucleotide Supplementation: Understanding Metabolic Variability and Clinical Implications, *Metabolites* 14 (2024) 341.
- [63] D. Yu, Q. Luo, C. Yang, C. Yang, S. Li, Z. Wang, Q. Wang, W. Liu, H. Wang, D. Ji, Electrostatic complexes of ethyl lauroyl arginate/nano-montmorillonite as a food-grade pickering stabiliser: Emulsification performance and mechanism, *LWT* 168 (2022) 113934.
- [64] D. Yu, Q. Luo, J. Zhang, Q. Wang, H. Wang, Z. Song, S. Li, W. Liu, F. Zhang, D. Ji, Pickering emulsions co-stabilised by cellulose nanofibres and nicotinamide mononucleotide, *Cellulose* 29 (2022) 8569–8585.
- [65] Z. Li, D. Yu, Formulation and characterization of pH-responsive pickering emulsions stabilized by soy protein isolate/nicotinamide mononucleotide complexes for controlled drug release, *Ind. Crop. Prod.* 203 (2023) 117158.
- [66] Z. Li, R. Zhao, F. Hu, Y. Zhang, B. Dong, P. Lu, Z. Song, H. Wang, F. Zhang, W. Liu, D. Yu, H. Li, Pickering emulsions stabilized by whey protein isolate/nicotinamide mononucleotide complex and their application in pH-responsive drug delivery, *Ind. Crop. Prod.* 214 (2024) 118494.
- [67] R. Xu, Electrophoretic Light Scattering, in: B. Scarlett (Ed.), *Particle Characterization: Light Scattering Methods*, Springer, Netherlands, Dordrecht, 2002, pp. 289–343.
- [68] A. Sze, D. Erickson, L. Ren, D. Li, Zeta-potential measurement using the Smoluchowski equation and the slope of the current-time relationship in electroosmotic flow, *J. Colloid Interface Sci.* 261 (2003) 402–410.
- [69] F.J. Carrión, A. De La Maza, J.L. Parra, The Influence of Ionic Strength and Lipid Bilayer Charge on the Stability of Liposomes, *J. Colloid Interface Sci.* 164 (1994) 78–87.
- [70] A.M. Holsaeter, K. Wiggard, I. Karlén, J.F. Hemmingsen, M. Brandt, N. Skalko-Basnet, How docetaxel entrapment, vesicle size, zeta potential and stability change with liposome composition-A formulation screening study, *Eur. J. Pharm. Sci.* 177 (2022) 106267.
- [71] L. Jin, C.W. Jarand, M.L. Brader, W.F. Reed, Angle-dependent effects in DLS measurements of polydisperse particles, *Meas. Sci. Technol.* 33 (2022) 045202.
- [72] F.Z. Chen, N.A. Mauro, S.M. Bertrand, P. McGrath, L. Zimmer, K.F. Kelton, Breakdown of the Stokes-Einstein relationship and rapid structural ordering in CuZrAl metallic glass-forming liquids, *J. Chem. Phys.* 155 (2021) 104501.
- [73] J. Wang, W. He, L. Cheng, H. Zhang, Y. Wang, C. Liu, S. Dong, W. Zha, X. Kong, C. Yao, X. Li, A modified thin film method for large scale production of dimeric artesunate phospholipid liposomes and comparison with conventional approaches, *Int. J. Pharm.* 619 (2022) 121714.
- [74] H. Elsans, T.O.B. Olusanya, J. Carr-Wilkinson, S. Darby, A. Faheem, A.A. Elkordy, Evaluation of novel cationic gene based liposomes with cyclodextrin prepared by thin film hydration and microfluidic systems, *Sci. Rep.* 9 (2019) 15120.
- [75] F. Quemeneur, M. Rinaudo, B. Pépin-Donat, Influence of Polyelectrolyte Chemical Structure on their Interaction with Lipid Membrane of Zwitterionic Liposomes, *Biomacromolecules* 9 (2008) 2237–2243.
- [76] I. van Uitert, S. Le Gac, A. van den Berg, The influence of different membrane components on the electrical stability of bilayer lipid membranes, *Biochimica et Biophysica Acta (BBA) Biomembranes* 1798 (2010) 21–31.
- [77] M.R. Prausnitz, R. Langer, Transdermal drug delivery, *Nat. Biotechnol.* 26 (2008) 1261–1268.
- [78] A. Simon, M.I. Amaro, A.M. Healy, L.M. Cabral, V.P. de Sousa, Comparative evaluation of rivastigmine permeation from a transdermal system in the Franz cell using synthetic membranes and pig ear skin with in vivo-in vitro correlation, *Int. J. Pharm.* 512 (2016) 234–241.
- [79] D. Seo, A.M. Schrader, S.Y. Chen, Y. Kaufman, T.R. Cristiani, S.H. Page, P. H. Koenig, Y. Gizaw, D.W. Lee, J.N. Israelachvili, Rates of cavity filling by liquids, *PNAS* 115 (2018) 8070–8075.
- [80] H. Xing, X. Pan, Y. Hu, Y. Yang, Z. Zhao, H. Peng, J. Wang, S. Li, Y. Hu, G. Li, D. Ma, High molecular weight hyaluronic acid-liposome delivery system for efficient transdermal treatment of acute and chronic skin photodamage, *Acta Biomater.* 182 (2024) 171–187.
- [81] G. Poornima, M. Deepa, M. Devadharshini, G. Gopan, M. Mani, S. Kannan, In-situ synthesis and evaluation of anti-bacterial efficacy and angiogenesis of curcumin encapsulated lipogel dermal patch for wound healing applications, *Biomaterials Advances* 164 (2024) 213989.
- [82] D.G. Amisha, G., Singh, H., Singh, S., and Singh, A., QbD-assisted optimisation of liposomes in chitosan gel for dermal delivery of aceclofenac as synergistic approach to combat pain and inflammation, *Drug Deliv. Transl. Res.* 14 (2024) 2403–2416.
- [83] E.A. López-Guajardo, R. Galluzzi, F. Delgado-Licona, R. Morales-Menendez, Process intensification of a catalytic-wall Taylor-Couette reactor through unconventional modulation of its angular speed, *Chem. Eng. J.* 489 (2024) 151174.