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Development of Liposomal and Polymeric Nanocarriers for Luteolin Delivery: A Senolytic-Oriented Approach †

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Abstract

Luteolin is a naturally occurring flavonoid with growing interest for its senolytic properties. However, its poor water solubility and low bioavailability limit clinical application. This study aimed to develop and compare two types of nanocarriers, liposomes and polymeric nanoparticles, for the efficient delivery of luteolin in senolytic therapies. Liposomes with luteolin were prepared using the lipid film hydration method, followed by sonication and extrusion. Polymeric nanoparticles were developed via the nanoprecipitation method using pullulan acetate, a hydrophobic derivative obtained by chemical functionalization of pullulan. Pullulan was biosynthesized over 72 h using the microorganism Aureobasidium pullulans ICCF 36 (from CMII–INCDCF-ICCF). The formulation used a polymer-to-luteolin ratio of 10:1 (g/g) and Pluronic F127 as a stabilizer. Nanoprecipitation was carried out under controlled conditions: stirring at 700 rpm and dropwise addition at 0.5 mL/min. Luteolin was successfully encapsulated in both delivery systems. Liposomes showed an encapsulation efficiency of 85.07 ± 0.09% and nanoscale diameter. Polymeric nanoparticles demonstrated an encapsulation efficiency of 74.87 ± 0.05%, nanometric size and a formulation yield of 73.29 ± 0.09%. Both liposomal and polymeric nanoparticle systems effectively encapsulated luteolin, with high efficiency and yield. The formulations present promising potential for use in senolytic therapies, targeting age-related cellular dysfunction. Further studies will assess their release kinetics, biological activity, and senolytic effects in vitro and in vivo.

Keywords: luteolin; liposomes; polymeric nanocarriers; senolytic

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1. Introduction

Cellular senescence, a permanent state of cell cycle arrest triggered by damage or aging, plays a central role in the progression of age-related disorders. The accumulation of senescent cells contributes to tissue dysfunction, chronic inflammation, and impaired regenerative potential, highlighting the need for strategies that selectively target these cells [1,2]. Senolytic therapies, which aim to eliminate senescent cells, have emerged as a

promising approach to mitigate aging-associated pathologies and improve tissue homeostasis [3].

Luteolin, a naturally occurring flavonoid present in various fruits, vegetables, and medicinal plants, has recently gained attention for its potential senolytic activity. It exhibits multiple bioactivities, including antioxidant, anti-inflammatory, and anti-proliferative effects, positioning it as a candidate for interventions targeting senescence [4,5]. Nevertheless, its clinical application is limited by poor aqueous solubility and low bioavailability, which restrict effective delivery to target tissues [6].

Nanocarrier-based delivery systems offer a viable solution to these limitations. By encapsulating luteolin in nanostructures, it is possible to enhance solubility, protect the compound from degradation, and enable controlled release. Liposomes, consisting of phospholipid bilayers, are highly biocompatible and can accommodate both hydrophilic and hydrophobic molecules, whereas polymeric nanoparticles provide tunable physical properties, high encapsulation efficiency, and prolonged release profiles [7–9].

In this study, we developed and compared two nanocarrier systems for luteolin delivery in senolytic applications: liposomes and polymeric nanoparticles. Liposomes were prepared using the lipid film hydration method with subsequent sonication and extrusion, while polymeric nanoparticles were synthesized via nanoprecipitation using pullulan acetate, a hydrophobically modified derivative of pullulan produced by *Aureobasidium pullulans* ICCF 36. Both formulations were evaluated for encapsulation efficiency, particle size, and formulation yield, aiming to identify a nanocarrier platform suitable for enhancing luteolin delivery and its senolytic potential.

2. Materials and Methods

2.1. Materials

Pluronic F127 (poly(ethylene glycol)-block-poly(propylene glycol)-block-poly(ethylene glycol)), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), diammonium sulfate ($(NH_4)_2SO_4$), and sodium nitrate ($NaNO_3$), phosphatidylcholine (from egg yolk), luteolin were obtained from Sigma-Aldrich. Methanol (HPLC grade), ethanol (HPLC grade), acetone (analytical grade) was purchased from AdraChim SRL (Bucharest, Romania) and used as received. Distilled water was used for all experimental procedures.

2.2. Pullulan Biosynthesis and Pullulan Acetate Preparation

Pullulan was biosynthesized using Aureobasidium pullulans ICCF 36 (Collection of Microorganisms of Industrial Importance, INCDCF-ICCF). The biosynthesis process was conducted under submerged cultivation conditions to obtain extracellular polysaccharides with optimal yield and purity. For inoculum preparation, the strain was first cultured on YMPG medium (glucose 1%) to avoid pigment formation, as colonies grown on Ap agarized medium produced a dark green–black pigment inversely correlated with polysaccharide synthesis. A 1% (*v*/*v*) inoculum was then transferred into the biosynthesis medium consisted of glucose (8%), $NaNO_3$ (0.2%), $(NH_4)_2SO_4$ (0.2%), KH_2PO_4 (0.5%), NaCl (0.2%), and $MgSO_4 \cdot 7H_2O$ (0.08%). Fermentation was carried out at 28 °C under continuous agitation at 220 rpm for 72 h. After 72 h of fermentation, the mean crude polysaccharide content obtained was 8.78% (*w*/*v*). The fermentation yield was approximately 1.51 g of polysaccharide per g of glucose consumed, demonstrating efficient substrate conversion under the selected culture conditions. Following microbial synthesis, the fermentation broth was centrifuged to remove cells and insoluble residues. The crude polysaccharide present in the supernatant was precipitated by adding three volumes of cold ethanol,

washed several times with distilled water, and dried under vacuum to obtain purified pullulan.

The purified pullulan was chemically modified to obtain pullulan acetate. Acetylation was performed in dimethylformamide (DMF) using pyridine as a catalyst and acetic anhydride as the acetylating agent. The reaction mixture was magnetically stirred for 24 h at room temperature. The resulting product was precipitated in cold distilled water, filtered, washed thoroughly to remove unreacted reagents, and dried at 40 °C. The obtained pullulan acetate was subsequently used as the polymeric matrix for nanoparticle formulation.

2.3. Preparation of Luteolin-Loaded Polymeric Nanocarriers

Polymeric nanoparticles loaded with luteolin were prepared by the nanoprecipitation technique using pullulan acetate as a biodegradable polymer matrix and Pluronic F127 as a stabilizer. Acetone (5 mL) was used to dissolve 50 mg of pullulan acetate. Luteolin was precisely weighed and dissolved in the pullulan acetate/acetone solution at a polymer-to-drug ratio of 10:1 (*w*/*w*). The resulting organic phase was added dropwise at a rate of 0.5 mL/min into the aqueous phase (either distilled water or a 0.3% Pluronic F127 aqueous solution) under continuous magnetic stirring at 700 rpm and ambient temperature (25 °C). The mixture was stirred until complete evaporation of the organic solvent, allowing the formation of luteolin-loaded polymeric nanoparticles. Following synthesis, the nanoparticle suspensions were centrifuged at 10,000 rpm for 30 min to remove unencapsulated luteolin and other impurities. The purified nanoparticles were subsequently washed with distilled water and re-dispersed for further characterization.

2.4. Preparation of Liposomes Loaded with Luteolin

Luteolin-loaded liposomes were produced using a combination of thin-film hydration, sonication, and extrusion techniques. Briefly, luteolin (20 mg) and phosphatidylcholine (100 mg) were dissolved in 10 mL of ethanol. The mixture was allowed to stand at room temperature for three hours to facilitate phospholipid swelling. A thin and uniform lipid film was subsequently formed by evaporating the organic solvent at 35 °C under reduced pressure using a rotary evaporator (Laboranta 4000, Heidolph Instruments GmbH & Co. KG, Kelheim, Germany). The resulting lipid film was hydrated with bidistilled water at 35 °C to stabilize the vesicles, and the suspension was maintained at 25 °C for two hours. The lipid suspensions were then subjected to probe sonication for 20 min at 50% amplitude (Sonorex-Digital-10P, Bandelin Electronic, Berlin, Germany) to reduce vesicle size. Subsequently, the suspension was extruded five times through polycarbonate membranes with pore sizes of 0.4 μm and 0.2 μm to achieve homogeneous nanoscale dispersion. Unencapsulated luteolin was removed by centrifugation at 12,000 rpm for 20 min at 5 °C, and the pellet containing the encapsulated vesicles was resuspended in distilled water. The formulation was prepared in triplicate and stored at 4 °C until further characterization.

2.5. Characterisation of Liposomal and Polymeric Nanocarriers Loaded with Luteolin

Liposomal and polymeric nanocarriers were evaluated for encapsulation efficiency, formulation yield, polydispersity index (PDI), average particle size, and stability. Encapsulation efficiency (EE) was calculated as the percentage of senotherapeutic successfully incorporated into nanoparticles relative to the initial amount used. The encapsulated drug content was quantified via UV–Vis spectrophotometry (Helios, ThermoFisher Scientific, Waltham, USA) at 350 nm using a calibration curve for luteolin ($y = 0.0625x + 0.00534$; $R^2 = 0.9642$).

The production yield was determined as the mass ratio of obtained nanoparticles to the total mass of starting materials. Particle size and PDI were measured using dynamic light scattering (Beckman-Coulter N4-PCS Submicron, Paris, France). To minimize multiple scattering effects, nanoparticles were diluted 1:10 with distilled water prior to measurement. Measurements were performed at room temperature with ten replicates per sample.

Nanoparticle stability was assessed over a three-month period by storing samples in amber glass vials at 4 °C and monitoring changes in encapsulation efficiency at 0, 1, 2, and 3 months.

2.6. Statistical Analysis

All experiments were performed in triplicate. Data are expressed as mean ± standard deviation (SD). Differences were considered statistically significant at $p < 0.05$.

3. Results and Discussion

Luteolin was successfully encapsulated in both liposomal and polymeric nanoparticle delivery systems, demonstrating the suitability of these nanocarriers for bioactive flavonoid delivery; their characteristics are displayed in Table 1. Liposomes exhibited a high encapsulation efficiency of 85.07 ± 0.09%, with nanoscale particle size, confirming their capability to efficiently incorporate luteolin within the lipid bilayer. Polymeric nanoparticles also effectively encapsulated luteolin, with an encapsulation efficiency of 74.87 ± 0.05%, nanometric size, and a formulation yield of 73.29 ± 0.09%, indicating efficient drug loading and production reproducibility. The stability of the nanosystems loaded with luteolin are presented in Table 2.

The high encapsulation efficiency observed in both systems suggests strong interactions between luteolin and the carrier matrices. Liposomes, due to their phospholipid bilayer structure, likely provided a favorable hydrophobic environment for luteolin, whereas polymeric nanoparticles facilitated encapsulation through polymer–drug interactions and matrix entrapment. The slightly lower encapsulation efficiency in polymeric nanoparticles may be attributed to differences in polymer composition, drug–polymer affinity, or preparation parameters.

Table 1. Characteristic of liposome and nanoparticles loaded with luteolin.

Sample	EE (%)	Size (nm)	PDI	Yield
Liposomes@Luteolin	85.07 ± 0.09%	207.5± 3.44	0.218	84.19 ± 0.07%
NP_PA@Luteolin	74.87 ± 0.05%	219.9 ± 1.55	0.217	73.29 ± 0.09%

Table 2. Stability of liposome and nanoparticles loaded with luteolin.

Sample	EE (%) Initial	EE (%) 1 Months	EE (%) 2 Months	EE (%) 3 Months
Liposomes@Luteolin	85.07 ± 0.09%	84.05 ± 0.01%	83.88 ± 0.05%	83.57 ± 0.01%
NP_PA@Luteolin	74.87 ± 0.05%	74.09 ± 0.01%	73.17 ± 0.04%	72.81 ± 0.04%

Both delivery systems present promising potential for senolytic therapies, aimed at targeting age-related cellular dysfunction. The nanoscale size of the formulations is advantageous for enhanced cellular uptake and bioavailability, while the high encapsulation efficiency ensures sufficient drug payload for therapeutic effects. These findings lay the groundwork for subsequent studies to investigate release kinetics, biological activity, and senolytic efficacy in vitro and in vivo, which are critical for evaluating their potential translational application in age-related interventions.

In conclusion, both liposomal and polymeric nanoparticle formulations demonstrate effective luteolin encapsulation, favorable physicochemical characteristics, and potential as delivery platforms for senolytic therapy, supporting further exploration in preclinical models.

4. Conclusions

This study successfully developed and compared two nanocarrier systems, liposomes and polymeric nanoparticles, for the delivery of luteolin, a flavonoid with potential senolytic properties. Both delivery systems demonstrated high encapsulation efficiency, 85.07 ± 0.09% for liposomes and 74.87 ± 0.05% for polymeric nanoparticles, along with nanoscale particle size and, in the case of polymeric nanoparticles, a formulation yield of 73.29 ± 0.09%. These results highlight the effectiveness of the chosen preparation methods and the suitability of the carriers for luteolin encapsulation.

The liposomal and polymeric nanoparticle formulations show promising potential for senolytic therapies, offering advantages such as enhanced bioavailability and efficient drug delivery to target age-related cellular dysfunction. Future investigations will focus on evaluating release kinetics, biological activity, and senolytic efficacy in vitro and in vivo, which are essential for advancing these nanocarriers toward clinical application. Overall, the study demonstrates that both nanocarrier systems are viable platforms for luteolin delivery, supporting their further development in anti-aging therapeutic strategies.

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