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Form 3

Abstract of Thesis

Name （ Ni'matul IZZA ）	
Title	Studies on Characterization of Nanostructured Lipid Carrier and Its Applications to Encapsulation of Biomolecules (ナノ構造脂質担体の特性解析ならびに生体分子封入への応用に関する研究)

Abstract of Thesis

Nanostructured Lipid Carrier (NLC) is one of the most promising lipid nanoparticles. The combination of solid and liquid lipids used as the core region results in some beneficial features that can overcome the disadvantages relating to the target drugs and its precursors, such as instability and insolubility. In this study, a comprehensive characterization of physicochemical properties and internal structure of the NLC was performed. Based on the characterized properties of the NLC, a method to encapsulate biomolecules (i.e., polyphenols and RNA) was established, focusing on their loading performance and behaviors. The findings obtained are expected to be applied in optimizing the design and formulation of NLC as a drug carrier.

In chapter II, the self-assembly properties of NLC have been systematically characterized. A ternary diagram of the aqueous solution mixing three lipids (cetyl-palmitate (CP: solid), caprylic triglyceride (CaTG: liquid), and Tween 80(T80)) was clarified based on the properties to summarize the possible structures formed at different compositions of NLC, showing the formation of four possible NLC, such as micelle, O/W emulsion, solid-lipid nanoparticles, and their intermediate states. The formation of CP/CaTG/T80 assemblies is dominated by O/W emulsion-like characteristics, which were strongly affected by the presence of liquid lipid. Based on the ternary diagram, T80 was the most influential component in the NLC system. The above strategy to characterize the NLC self-assembly can provide us a fundamental and meaningful insight relating to the encapsulation of biomolecules.

In chapter III, a comprehensive characterization of the core region of the NLC was conducted, focusing on the molecular behaviors and their physicochemical properties. A modified method of conventional fluorescence-based analysis was newly developed to investigate the anisotropy of inner and outer cores of different NLCs. The obtained results show that NLC represented a unique core-shell structure with higher rigidity of the outer core than that of the inner core. Further internal characterizations revealed that NLC could have a polymorphism crystal lamellar structure depending on the lipid composition. The above findings improve the understanding of the microscopic structure and properties of NLC cores and could be applied to designing a specific NLC carrier for various products and administration.

The above studies clarify the dependence of lipid composition on the molecular and physicochemical properties of NLCs. Based on the characterized properties of NLC cores, a method to encapsulate some biomolecules was proposed based on the loading performance with its appropriate quality, by selecting polyphenols and tRNA as case studies of micro- and macro-biomolecules.

In chapter IV, three different polyphenols with different lipophilicity were employed as targeted loading drugs in NLC. The results indicated that the hydrophobicity of polyphenols could be the most influential on the entrapment efficiency of each polyphenol and further on the actual antioxidant activity. Resveratrol, the most hydrophobic molecule in this study, demonstrated the highest entrapment efficiency; however, it has the lowest specific antioxidant activity compared to other polyphenols, especially quercetin, the least hydrophobic molecules. Additionally, NLCs have improved all the antioxidant activity per unit concentration of polyphenol (specific antioxidant activity) compared to the free polyphenols. It was found that the antioxidative activity was varied depending on the plausible location of polyphenols in NLC core and the antioxidative activity could be varied depending on the properties of the employed polyphenols.

In chapter V, the evaluation of NLC performance in loading RNA-based products is conducted. tRNA was selected as a product model and 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP) was employed as a supporting lipid to improve its loading in NLCs. The effect of lipid composition as well as the DOTAP concentration will be investigated by analyzing the circular dichroism (CD) spectroscopic analysis. The conformation of tRNA was evaluated with circular-dichroism spectroscopy by varying the timing of the tRNA addition of precursor solution of NLC. It was found that the DOTAP-modified NLC with its appropriate characters for encapsulation could entrap the tRNA at high capacity and could maintain the tRNA conformation without unmarkable change.

In chapter VI, the general conclusion of this study is summarized, and suggestions for clarifying the role of NLC as a potential drug delivery system and its wide application in many drug administrations are discussed as an extension of this thesis.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

新たな薬剤送達担体としてナノ構造脂質担体(NLC)が注目されている。NLCは、性状の異なる脂質(例 固体脂質や液体脂質(常温))と界面活性剤から構成される分子集合体であり、近年、抗がん医薬品送達やCOVID-19ワクチンなどのキャリアとしてNLCを活用する例が顕著に増加している。しかし、現時点NLC研究は、(i)調製方法(脂質種類・組成の探索など)や(ii)前述の応用研究(例 DDSキャリア、機能性食品など)に終始しており、定性的な知見を述べた報告が多数を占める状況である。NLCそのものの性質・特性を解析し、網羅的・体系的な解析データに基づいて、合目的的・合理的に設計する手法に関する研究成果はほとんど報告されていない。本学位論文では、各種生体分子を封入するためのナノ構造脂質担体(NLC)の新規な設計方法が提案されている。第1章では、NLCを含めた薬剤分子担体に関する背景を調査した。第2章では、Caprylic Triglycesides (CaTG: 液体脂質)、Cetyl Palmitate (CP: 固体脂質)、Tween 80 (T80：界面活性剤)の混合物から調製されるNLCに着目し、その物理化学的な評価方法を確立した。三角線図上で組成条件を系統的に変化させたNLCを調製し、ダブル蛍光プローブ法(DPH, Laurdan)を用いて、各種のNLCの集合形態(ミセル、SLN(Solid Lipid Nano-Particle), O/W Emulsion(NLC)ほか)を判別する手法を確立した。CaTG:CP比率を変えて調整した3種類のNLC)に着目したところ、同じNLCでも異なる表面物性を示すことを明らかにした。第3章では、修正蛍光プローブ法(DPH)を用いて、NLCの外郭部分と内部部分の特性を解析し、内外で異なる物性値を有することを示した。さらには、示差走査熱量分析(DSC)、cryo-TEM分析、XRD分析結果に基づいて、NLCが単なる油滴ではなく、コア-シェル型の構造を有し、かつ、特定の条件では、内部にラメラ構造を有することを初めて示した。以上の2-3章の結果に基づき、NLC調製条件を探索し、定量的な評価指標に基づいて、内部構造・特性を設計する手法をスキームとしてまとめた。そのケーススタディとして、第4章では、低分子量分子(ポリフェノール類)、第5章では、高分子量分子(tRNA)を内封するための条件を明らかにして、得られた生体分子封入NLCの性能を評価することで、提案した手法の妥当性を示した。今後、DDSキャリアデザインや機能食品製造のための指針となる手法であると考えられる。以上の理由により、本論文を博士（工学）の学位論文として価値のあるものと認める。