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科学研究費助成事業　　研究成果報告書

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研究課題名（和文）Development of supercritical carbon dioxide mediated microparticles of juvenile hormone analogues (JHAs) for the elimination of mosquito-borne diseases

研究課題名（英文）Development of supercritical carbon dioxide mediated microparticles of juvenile hormone analogues (JHAs) for the elimination of mosquito-borne diseases

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交付決定額（研究期間全体）：（直接経費）　　3,100,000円

研究成果の概要（和文）：超臨界二酸化炭素を介した幼若ホルモン類縁体（JHAs）の微粒子化法の開発に関する私の研究は、昆虫害虫管理、グリーンケミストリー、農業の持続可能性の分野に大きく貢献しました。私たちの研究成果は、4つの国内・国際会議で発表され、知識を広め、研究に対するフィードバックを受けるために、同業者や専門家と交流した。Wiley誌（Asia-Pacific J. of Chem. Eng.(https://doi.org/10.1002/apj.3043)に掲載されました。また、J. of the Ins. of Ind. App. Eng.（10.12792/JIIAE.10.60）に掲載された。

研究成果の学術的意義や社会的意義
JHAのscCO2媒介微粒子に関する私の研究は、薬剤製剤に持続可能な溶媒を使用することでグリーンケミストリーを発展させるものです。粒子工学を革新し、JHAの性能とバイオアベイラビリティを最適化することで、効果的な害虫駆除を実現します。社会的には、化学汚染を減らすことで環境の持続可能性を促進し、経済的に実行可能で安全な農法をサポートします。これにより、食品の安全性、消費者の信頼、そして人々の健康を向上させ、世界的な持続可能性の目標に沿うとともに、持続可能な農業に対する人々の意識を高めています。

研究成果の概要（英文）：My research on the development of supercritical carbon dioxide-mediated microparticles of juvenile hormone analogues (JHAs) methods has made significant contributions to the field of insect pest management, green chemistry, and agricultural sustainability. Our research findings were presented at 4 national and international conferences and engaged with peers, and experts to disseminate the knowledge and receive feedback on the research. Presentation in the ASCON-IEEChE 2023 KOREA (2023, 12), ICSST23, Okinawa 2023,11, 化学工学会 第53回秋季大会 (2022, 9) and in the ICCCI (2022,11) were highly evaluated. Two articles were published in the Journal of Wiley : Asia-Pacific J. of Chem. Eng.(https://doi.org/10.1002/apj.3043). And, in the J. of the Ins. of Ind. App. Eng. (10.12792/JIIAE.10.60).

研究分野：Green Chemistry and Sustainable Agriculture

キーワード：supercritical CO2　JHA　Pyriproxyfen　microencapsulation　control release　two-phase separation

１．研究開始当初の背景

Mosquitoes are the deadliest animals on the world, transmitting deadliest mosquito-borne diseases like malaria, yellow fever, dengue etc. Among them about 390 million infection cases causes dengue each year worldwide, with 25,000 deaths, mostly in children (WHO, 2019). This data already told us that present mosquito control strategies are insufficient and undoubtedly failed to eliminate mosquito-borne diseases. The agricultural sector faces challenges in pest management, with traditional chemical pesticides posing environmental and health risks. Juvenile hormone analogues (JHAs) have emerged as promising alternatives due to their targeted action on insect growth regulation. However, their formulation and delivery remain challenging. Supercritical carbon dioxide ($scCO_2$) has gained attention as a green solvent for drug formulation, offering advantages in sustainability and particle engineering. This research aims to develop a novel method for synthesizing $scCO_2$-mediated microparticles of JHAs, aiming to enhance their bioavailability, stability, and efficacy in insect pest management.

２．研究の目的

The primary purpose of this research is to develop a sustainable and effective method for the formulation of juvenile hormone analogues (JHAs) using supercritical carbon dioxide ($scCO_2$) as a solvent and antisolvent. The research aims to:

1. Innovate green chemistry methods for pesticide formulation.
2. Optimize particle engineering to control the size, morphology, and drug loading of JHA microparticles.
3. Evaluate the stability, and insecticidal efficacy of $scCO_2$-mediated JHA microparticles for enhanced insect pest management.

３．研究の方法

The research methodology involves:

Synthesis of $scCO_2$-mediated microparticles of JHAs using a supercritical fluid technology.

1.Characterization of the microparticles for particle size, morphology, drug loading, and stability.

2.Evaluation of the bioavailability and insecticidal efficacy of the $scCO_2$-mediated JHA microparticles through laboratory assays and field trials.

3.Comparison of the developed method with traditional formulation techniques to assess its advantages in sustainability, efficiency, and effectiveness.

４．研究成果

1. Melting Point Depression of PCL in the Presence of CO_2

Supercritical carbon dioxide ($scCO_2$) acted as a plasticizing agent, reducing the melting point (Tm) of polycaprolactone (PCL) from 60.0 °C to 45.0 °C under 10 MPa pressure. This reduction in Tm is crucial due to the inter-batch and inter-manufacturer variability of commercially provided PCL. For the subsequent Pressure Gas-Expanded Liquid (PGSS) processing of PCL particles, an increment of approximately 5 °C was added to the melting temperature to ensure complete melting and avoid clogging.

2. Encapsulation and Characterization of Microparticles

Microparticle formation of Poly(propylene fumarate) (PPF) in PCL was achieved using PGSS with $scCO_2$ at pre-expansion pressures of 8–14 MPa and temperatures of 50–70 °C. Scanning Electron Microscopy (SEM) revealed varying morphological characteristics at different processing conditions (Fig. 1). Microparticles produced at 50–60 °C and 10 MPa exhibited

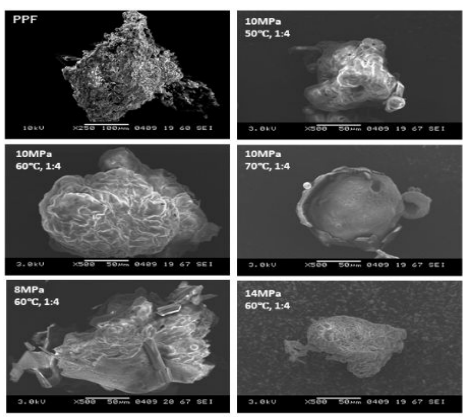

Figure 1 SEM images of PPF-PLC microparticles obtained from the PGSS process using $scCO_2$ solutions at different process parameters.

irregular shapes with creases, indicating internal strains during rapid expansion. Conversely, particles produced at 70 °C and 10 MPa were round-shaped due to decreased $scCO_2$ viscosity enabling faster mass transfer. Particle size distribution (PSD) measurements showed fine microparticles with diameter values varying between 47.7 and 186.3 µm. Optimized conditions were determined to be a minimum pressure of 10 MPa and a temperature between 50–60 °C for microencapsulation of PPF in PCL.

3. FT-IR Analysis

FT-IR spectra confirmed the presence of PPF and PCL in the microparticles, with no shifts observed, indicating successful encapsulation of PPF within the PCL without any drug-polymer interactions. The major peaks of PPF "as received" at 1600 cm-1 and 843 cm-1 were attributed to the stretching vibration of the aromatic C=C stretch and p-substituted benzene, respectively, and appeared in the PPF–PCL microparticles spectrum established the presence of PPF in the PGSS particles (Fig. 2).. PCL "as received" displays a large peak of the stretching vibration of free bonded carbonyls at 1720 cm-1, and the C–O stretch at 1500–1000 cm-1 were also observed in the PPF–PCL microparticles spectrum at the same position, and there was no shift of the absorbance observed after microencapsulation, suggesting successful encapsulation of PPF without any drug interaction within the polymer.

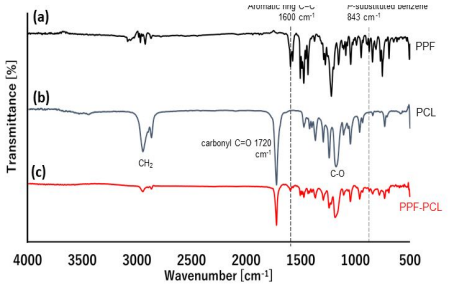

Figure 2 FTIR spectra of (a) PPF "as received", (b) PCL "as received", and (c) PPF-PCL microparticles

4. In-vitro Dissolution Studies

Dissolution studies (Fig. 3). revealed a slow and controlled release pattern of PPF from PPF-PCL microcapsules produced at 60 °C and 10 MPa, with approximately 4% of the total active ingredient released over 140 days. In the first 7 d, PPF in replaced water was released at a constant rate (36.0 ± 3.1 ppb/d), and the cumulative release was 252.3 ± 9.9 ppb, which was lower than the saturation level of PPF in water (367 ppb). The cumulative release of PPF for 140 d was calculated using the exponential approximation formula $Y = 51.444e^{0.0028x}$, where Y is the PPF concentration (ppb), and X is the number of days after treatment. The release kinetics followed zero-order kinetics, attributed to the smaller particle size and increased surface area of the microparticles, enhancing the diffusion of PPF.

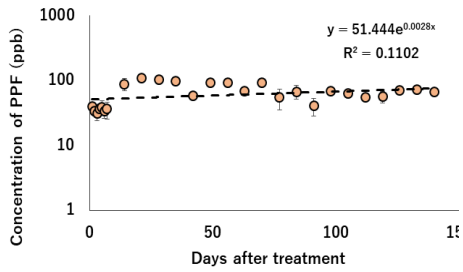

Figure 3 Changes in the concentration of PPF in daily replaced water treated with microparticles.

5. In-vivo Emergence Inhibition (EI) Bioassay

In-vivo studies (Fig. 4). using fourth instar larvae of Ae. albopictus demonstrated 95–100% inhibition of emergence, confirming the effectiveness of PPF-PCL microparticles in controlling mosquito populations. The slow release of PPF from the microparticles over the mosquito emergence period suggests potential applications in mosquito control, with the release rate being controlled by the biodegradable polymer PCL coating and $scCO_2$-mediated PGSS process.

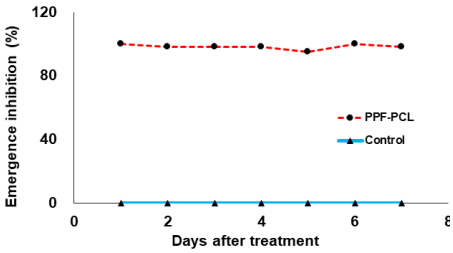

Figure 4 Changes in inhibition of emergence against fourth instars of Ae. albopictus with daily replaced water treated with PPF-PCL microparticles.

The research successfully demonstrated the formulation of PPF-PCL microparticles using $scCO_2$-mediated PGSS, showcasing the versatility of $scCO_2$ in reducing the melting point of PCL, optimizing particle size and morphology, and enabling controlled drug release. The environmentally friendly nature of the process, avoiding the use of toxic organic solvents and non-biodegradable substances, highlights the potential of this method in sustainable drug delivery and pest control applications. Further studies are warranted to explore the scalability, long-term stability, and field efficacy of these microparticles for practical applications.

５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　0件／うち国際共著　2件／うちオープンアクセス　2件）

１．著者名 Tanjina Sharmin, Hiroyuki Tashiro, Hiroki Sakai, Kouichiro Shibata, Konoka Ema, Keiichi Irie, Tomomitsu Satho, Kenji Mishima	４．巻 0
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掲載論文のDOI（デジタルオブジェクト識別子） 10.1002/apj.3043	査読の有無 無
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

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オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

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2021年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

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７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関