Discover Chemistry

Brief Communication

Effect of double coating on microencapsulation of levofloxacin using the particles from gas-saturated solutions process as a controlled-release system

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Received: 17 October 2024 / Accepted: 19 March 2025
Published online: 02 April 2025
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Abstract
A novel double-coating technique was employed to encapsulate levofloxacin (LVF), a potent antibacterial drug, using the particles from gas-saturated solutions (PGSS) process to achieve extended release. The primary coating utilized glyceryl tristearate (GT), a lipid with a high melting point (72 °C), followed by a secondary coating with trimyristin (TM), a lipid with a lower melting point (52 °C). For comparison, a single-coating approach was also explored, using the biocompatible polymer poly-(ε-caprolactone) (PCL). The resulting particles were characterized for their shape, size, and LVF encapsulation efficiency, with confirmation of LVF entrapment provided by Fourier transform infrared (FTIR) spectroscopy, and X-ray powder diffraction (XRD) analysis. The particles, with an average diameter of 95.3 ± 16.5 µm, exhibited an encapsulation efficiency of up to 92.1 ± 2.5%. Furthermore, in vitro release studies revealed that the double-coated microcapsules effectively suppressed the initial burst release and provided controlled, extended release of LVF (t_{30D} = 36.3%), demonstrating the efficiency of this encapsulation method for prolonged drug delivery.

Keywords Gas-saturated solutions · Supercritical fluids · Levofloxacin · Glyceryl tristearate · Trimyristin · Microparticles

Abbreviations:

APIs	Active pharmaceutical ingredients
CDDS	Controlled drug delivery systems
GT	Glyceryl tristearate
LVF	Levofloxacin
PGSS	Particles from gas-saturated solutions
PCL	Poly-(ε-caprolactone)

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$scCO_2$ Supercritical carbon dioxide
TM Trimyristin
XRD X-ray powder diffraction

1 Introduction

Levofloxacin (LVF), a broad-spectrum fluoroquinolone antibiotic, is extensively prescribed to treat a wide range of bacterial infections due to its exceptional oral bioavailability (~ 99%) [1, 2]. Despite its efficacy, LVF's therapeutic application is hindered by rapid systemic clearance, necessitating frequent dosing and rigorous monitoring, especially in patients with renal impairments [3, 4]. Globally, frequent dosing regimens are associated with lower patient adherence, which further exacerbates treatment challenges and impacts therapeutic success. Furthermore, the increasing prevalence of bacterial resistance to LVF underscores the urgent need for advanced drug delivery systems to enhance its clinical utility and long-term effectiveness [5].

Controlled drug delivery systems (CDDS) offer a promising solution by maintaining consistent drug levels over extended periods, thereby addressing the limitations of conventional LVF administration. By reducing the need for frequent dosing, CDDS improve patient compliance, mitigate the risks of adverse effects, and enhance the overall therapeutic efficacy of LVF [6–8]. Among various approaches, microencapsulation stands out as an effective technique to achieve controlled drug release by entrapping the drug within a protective coating that governs its release rate. However, conventional encapsulation methods, such as solvent evaporation and spray drying, rely heavily on organic solvents, raising environmental and safety concerns [9–13]. These methods also tend to produce particles with inconsistent sizes and structural defects, leading to challenges such as the "initial burst effect," where a significant amount of the drug is released immediately. This rapid release can diminish therapeutic efficacy, increase the risk of bacterial resistance, and cause potential toxicity. Addressing these issues is essential for improving LVF-based drug delivery systems.

The particles from gas-saturated solutions (PGSS) process has recently emerged as a sustainable and efficient method for microencapsulation [14–19]. This technique addresses key challenges of conventional encapsulation methods by eliminating the need for organic solvents, reducing environmental concerns, and enabling the formation of microparticles with controlled size and improved structural integrity. The PGSS process uses supercritical fluids, such as CO_2, to form particles without the use of organic solvents, making it an environmentally friendly approach. This technique allows for the formation of microparticles with tunable size and morphology by dissolving CO_2 into a carrier material under moderate pressure, which expands upon depressurization to stabilize the active pharmaceutical ingredients (APIs). However, a common issue with PGSS-derived microcapsules is the formation of surface cracks, particularly when a molten polymer precipitates around the drug. These cracks allow external medium to penetrate the capsules, leading to rapid drug release, which is often undesirable in controlled-release formulations [16, 17].

To mitigate the initial burst release and enhance the controlled-release profile of LVF, a double-coating strategy is employed (Fig. 1). It is anticipated that any cracks in the primary coating will be masked by the secondary polymer coating. Furthermore, any cracks that may occur in the primary polymer film are not expected to be continuous. The secondary coating will serve as an additional barrier, delaying solvent penetration and ensuring a more gradual release of the API. While double coating has been well-established in the context of tablet formulations [20], its application at the microencapsulation level is novel, and this research explores its potential for the first time in the context of LVF encapsulation using the PGSS process.

To validate the efficacy of the double-coating system, this study includes a comparative analysis with poly-(ε-caprolactone) (PCL), a widely used polymer in drug delivery research. Renowned for its biocompatibility and biodegradability, PCL is commonly employed in controlled-release systems due to its ability to sustain drug release over extended periods [18]. By comparing with PCL, this study highlights the double-coating system's superior encapsulation efficiency, reduced burst effect, and enhanced release profiles. The well-documented properties of PCL as a benchmark material ensure that this comparison provides meaningful validation for the proposed double-coating approach.

In this study, LVF is encapsulated using a combination of two lipids with complementary melting points—glyceryl tristearate (GT, melting point 72 °C) as the primary coating and trimyristin (TM, melting point 52 °C) as the secondary coating. The influence of co-solvents on the encapsulation efficiency of the single-coating system is initially examined, followed by an evaluation of the in vitro release profile of the of both single- double-coating system. The results are compared with a single-coating system using TM and poly-(ε-caprolactone) (PCL) to underscore the advantages of the

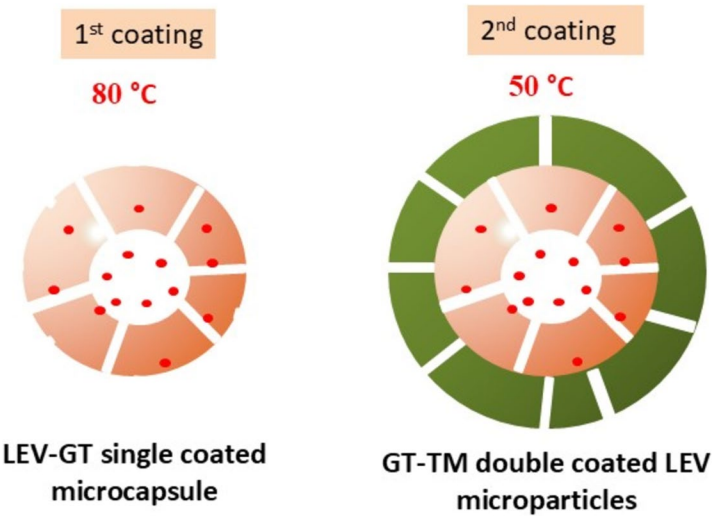

Fig. 1 Schematic of the double-coating concept via PGSS

double-coating strategy in addressing the limitations of conventional single-coating methods, paving the way for its broader application in controlled drug delivery systems.

2 Methods

2.1 Materials

Levofloxacin (MW: 361.37 g/mol, purity > 98.0 wt%, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), poly(ε-caprolactone) (MW: 10,000 g/mol, Aldrich Chemical Company, Inc., WI, USA), glyceryl tristearate (GT) (MW: 891.48 g/mol), and trimyristin (TM) (MW: 723.16 g/mol, IOI Oleo, Hamburg, Germany) (Fig. 2) were obtained and utilized without further processing. Additionally, CO_2 with a purity of > 99.9 vol.% (Fukuoka Sanso Co., Ltd., Fukuoka, Japan) and ethanol with a purity of > 99.5 wt% (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were purchased and used in their original form.

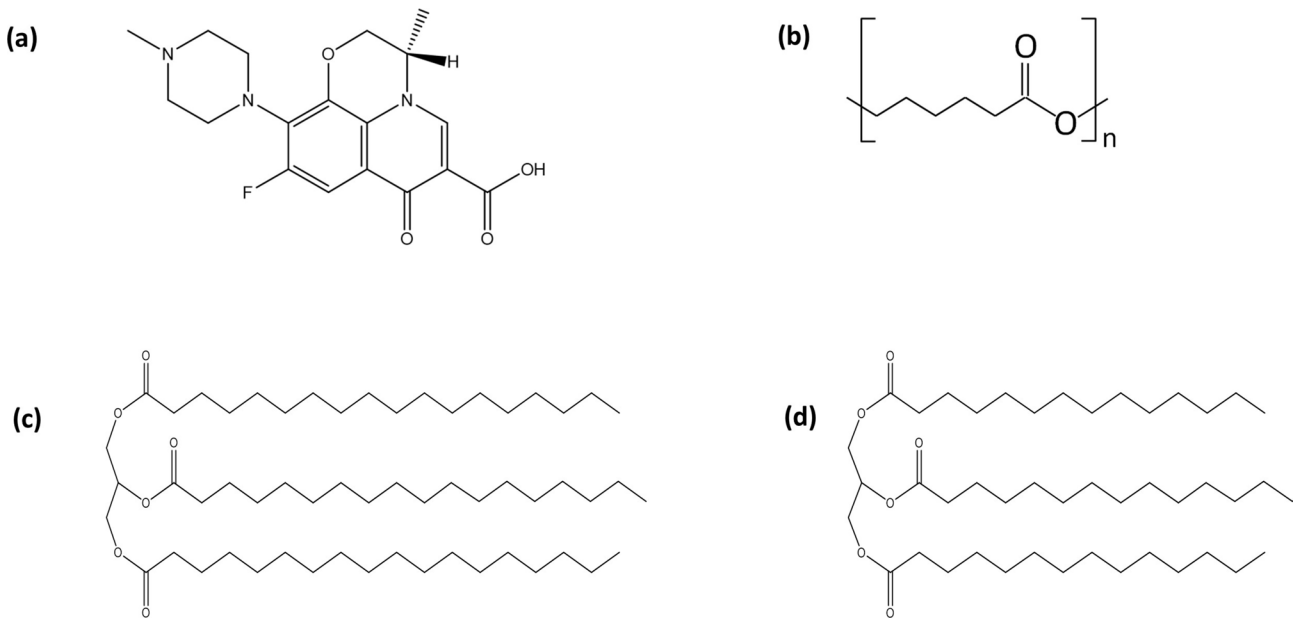

Fig. 2 Chemical structure of (**a**) Levofloxacin (LVF, MW: 3611.37 g/mol), (**b**) repeat unit of PCL, (**c**) glyceryl tristearate (GT, MW: 891.48 g/mol), and (**d**) trimyristin (TM, MW: 723.16 g/mol)

2.2 LVF -loaded microcapsule formation via PGSS

Figure 3 illustrates a schematic diagram of the experimental apparatus, as described in previous studies [16, 18]. Briefly, the experiment began with loading the active compound, levofloxacin (LVF), and the selected carrier (PCL, GT, or TM) into a preheated high-pressure cell (inner volume: 500 cm^3, Akico Co., Tokyo, Japan, SCV500A) at the specified drug–polymer ratios, which was then securely sealed. The high-pressure cell was submerged in a water bath maintained at a controlled temperature between 50 °C and 80 °C. Once the high-pressure cell reached the desired temperature, CO_2 was introduced to the target working pressure using a magnet pump (Iwaki Co., Tokyo, Japan). The pre-loaded materials were thoroughly mixed with CO_2 for 30 min to create a CO_2-saturated solution. Thereafter, the CO_2-saturated solution was depressurized and expanded into a chamber at atmospheric pressure, forming microcapsules via precipitation. Alternatively, the polymer can be saturated at lower temperatures by incorporating co-solvents, such as ethanol, which significantly aid in lowering the melting point of the polymer within a supercritical fluid environment. Experiments have shown that with a CO_2 impregnation of PCL has shown that the addition of 5–10% ethanol reduces the required processing temperature to around 35 °C, compared to ~ 55 °C without ethanol [21, 22]. Therefore, the co-solvent approach was employed, and to assess the influence of ethanol content on the encapsulation efficiency (EE, %) different amounts of ethanol (40–150 mL) were used. Table 1 provides an overview of all the process conditions investigated in this study, while Table 2 outlines the composition of the microcapsules, including the drug-to-excipient ratios. The operating pressure was maintained at 9 MPa and temperatures of the stirring chamber were either 60 °C for PCL, or 80 and 50 °C for GT and TM, respectively, depending on the melting point of the polymers. The values utilized in this study were determined based on insights gained from our earlier research on PCL microencapsulation via the PGSS process [7]. This prior work identified that the maximum melting

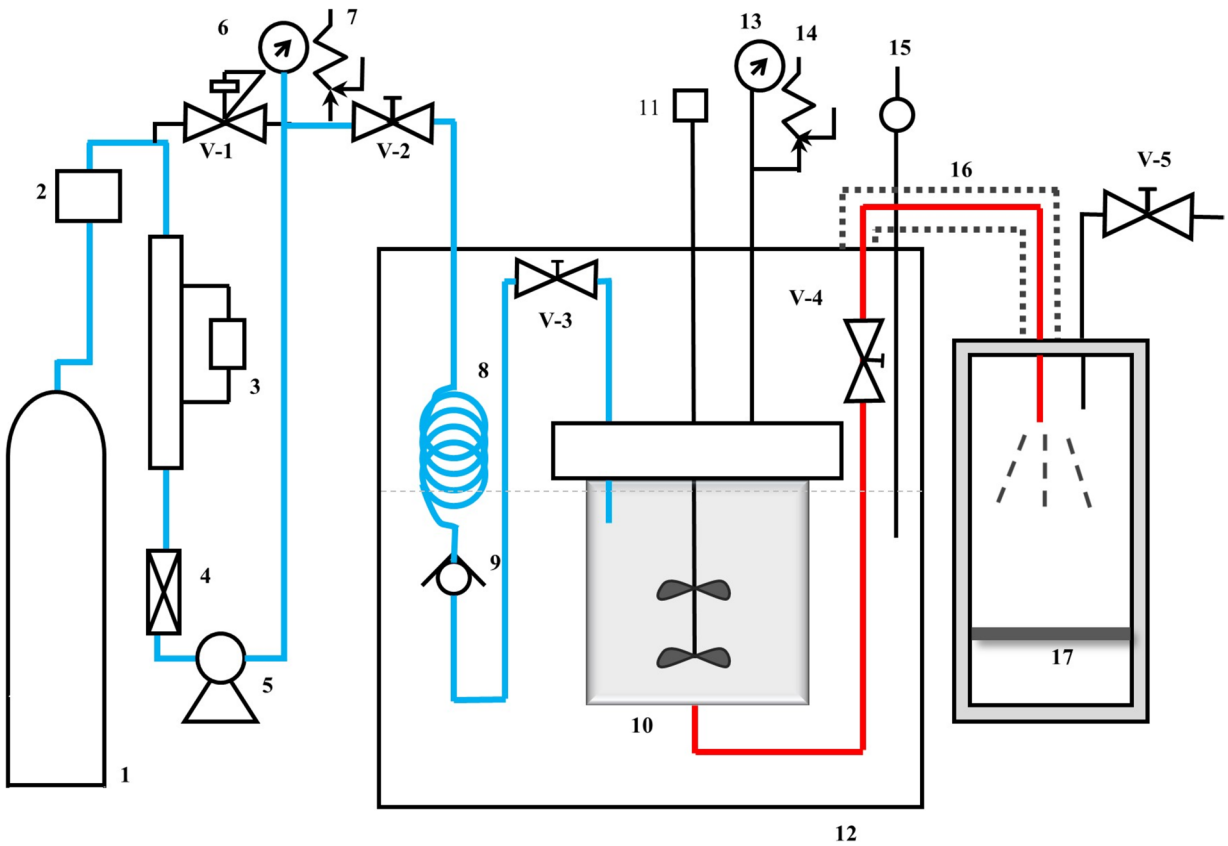

Fig. 3 Schematic diagram of apparatus used in the PGSS process. 1: gas cylinder; 2: dryer; 3: cooling unit; 4: filter; 5: pump; 6: pressure gauge; 7: safety valve; 8: preheater; 9: check valve; 10: high-pressure cell; 11: agitator; 12: water bath; 13: pressure gauge; 14: safety valve; 15: thermometer; 16: depressurization tunnel; and 17: atmospheric collector vessel with water. V-1 indicates a back-pressure regulator, and V-2 to -4 are stop valves

Table 1 Summary of operating conditions

Formulations			Ethanol	*T*	*P*	*Mean PSD*	$d_{0.1}$	$d_{0.5}$	$d_{0.9}$	Span	*EE*
1st	2nd		[g]	[℃]	[MPa]	(*μ*m) ± SEM	*μ*m	*μ*m	*μ*m		(%)
LVF:PCL	–	1:10	40	60	9	–	–	–	–	–	51.5
LVF:PCL	–	1:10	60	60	9	295.4 ± 37.9	114.5	256.0	468.1	1.38	81.2
LVF:PCL	–	1:10	80	60	9	–	–	–	–	–	75.2
LVF:PCL	–	1:10	120	60	9	–	–	–	–	–	72.3
LVF:PCL	–	1:10	150	60	9	–	–	–	–	–	51.3
LVF:GT	–	1:4	60	80	9	62.4 ± 13.8	5.6	28.0	140.0	4.79	89.3
LVF:GT	–	1:8	60	80	9						95.7
LVF-GT:	TM	1:4	60	50	9	95.3 ± 16.5	18.7	62.6	171.2	2.43	92.1

Data are presented as mean ± SEM (n = 3), where n represents the number of independent experimental replicates

Table 2 Composition of microcapsule formulations

Formulations	Loading ratio	*Drug* [g]	*PCL* [g]	*GT* [g]	*TM* [g]	Drug: Polymer	*Polymer: Polymer*	*EE*	*Thickness* [μm]
LVF:PCL	1:10	2.5	25			1:10	–	51.5	
LVF:PCL	1:10	2.5	25			1:10	–	81.2	81.28
LVF:PCL	1:10	2.5	25			1:10	–	75.2	
LVF:PCL	1:10	2.5	25			1:10	–	72.3	
LVF:PCL	1:10	2.5	25			1:10	–	51.3	
LVF:GT	1:4	1	-	4	-	1:4	–	89.3	12.9
LVF:GT	1:8	1		8	-	1:8	–	95.7	
LVF-GT/TM	1:4 (LVF-GT: TM)	0.18	-	0.82	4	1:27	1:5	92.1	31.9

point reduction caused by CO_2 occurred within the pressure range of 8–10 MPa, yielding the highest encapsulation efficiency (EE, %). The recovered microcapsules were subsequently collected and washed with 20 mL of 66.4% (v/v) solution of ethanol in water to remove any uncoated LVF and used for characterization and further studies. The concentration of free, unencapsulated LVF present in the aqueous-ethanol solution was quantified using a UV–Vis spectrophotometer at a wavelength of 290 nm. The encapsulation efficiency (EE, %) was calculated using Eq. (1).

$$EE\,(\%) = \left(W_1 - W_2\right)/W_1 \times 100 \tag{1}$$

Here, W_1 represents the total amount of LVF initially added to the solution prior to encapsulation, while W_2 corresponds to the amount of unencapsulated LVF remaining in the supernatant after centrifugation. The EE (%) thus reflects the proportion of LVF successfully encapsulated relative to the total initial amount. All experiments were performed in triplicates unless otherwise stated.

2.3 Product characterization

To assess the impact of the PGSS process, scanning electron microscopy (SEM, JSM6060, JEOL Ltd., Tokyo, Japan) was employed to evaluate the structural and morphological characteristics of the products before and after treatment. Particle size (PS) and particle size distribution (PSD) were determined using a laser diffraction particle size analyzer (SALD-2000, Shimadzu Co., Kyoto, Japan). The degree of polydispersity in the microparticles was quantified using the span value, calculated as:

$$\text{Span} = \frac{d0.9 - d0.1}{d0.5} \quad (2)$$

where $d_{0.1}$, $d_{0.5}$ and $d_{0.9}$ represent the particle diameters at 10%, 50%, and 90% of the cumulative distribution, respectively. Because of the similar properties of the drug (core) and coating polymer, it is difficult to determine the structure of the generated microparticles by X-ray analysis. Assuming that the drug and coating polymer form a uniform core–shell structure, the coating thickness of each particle was calculated geometrically from the measured average particle size and the drug and coating polymer composition.

Fourier transform infrared (FTIR) spectroscopy was employed to analyze the pure components as well as the single- and double-coated LVF microcapsules. The measurements were conducted using an attenuated total reflectance FTIR spectrometer (FT/IR-4600, JASCO, Tokyo, Japan) in transmission mode. Spectra were recorded over a scanning range of 500–4000 cm^{-1} at a controlled temperature of 25 °C.

The crystalline properties of the pure components and the single- and double-coated LVF microcapsules were analyzed using X-ray powder diffraction (XRD, Shimadzu XRD–6100, Kyoto, Japan). Diffraction patterns were obtained by measuring the X-ray intensity as a function of the diffraction angle (2θ) over a range of 10° to 50°.

2.4 LVF microcapsule in vitro release

The in vitro release of LVF from single- and double-coated microcapsules was evaluated using a vertical Franz-type diffusion cell (VIDTEK, Iwaki, Fukuoka, Japan) following established methods [16, 17]. A hydrophilic PTFE membrane (0.45 µm pore size, 65 µm thickness, 47 mm diameter; Merck Millipore, Tokyo, Japan) was placed between the donor and receptor chambers to prevent particle loss during sampling, ensuring controlled and reproducible drug release measurements. Unlike direct dispersion methods, which can lead to microcapsule loss and require time-consuming centrifugation, the Franz cell setup allowed for more consistent sampling while maintaining contact between the microcapsules and the release medium.

A measured 10 mg of microcapsules was applied to the membrane, and the receptor chamber was filled with 30 mL of phosphate buffer (pH 6.8) to maintain wetting and ensure controlled drug release. pH 6.8 was chosen because it closely mimics the intestinal environment, which is the primary site of absorption for orally administered drugs. While pH 7.4 is commonly used to simulate plasma conditions, pH 6.8 provides a more physiologically relevant medium for assessing drug dissolution and release from gastroretentive and enteric-coated formulations.

Sampling was conducted at predetermined intervals over 30 days to evaluate long-term stability and sustained-release behavior. Although drugs do not physically remain in the intestine for such an extended period, this timeframe was chosen to simulate prolonged drug release from extended-release formulations, a common practice for evaluating long-acting dosage forms such as implantable drug delivery systems [23]. This approach provides insights into the microcapsule's integrity, coating stability, and drug release kinetics over time, which are critical for developing controlled-release drug delivery systems.

The system was tightly sealed to prevent evaporation and shielded from light to protect LVF from photodegradation. Collected aliquots were analyzed at 290 nm using a UV–Vis spectrophotometer (JASCO, V-550, Tokyo, Japan), and the sampled medium was returned to maintain a constant receptor chamber volume.

3 Results and discussion

3.1 Encapsulation and characterizations of microparticles

The encapsulation of LVF using the PGSS process demonstrated promising results in terms of particle size, morphology, and encapsulation efficiency (EE). The PGSS single-coating process of LVF with PCL using $scCO_2$ solutions at 60 °C and 9 MPa resulted in spherical microparticles (Fig. 4a and 4b). with an average particle size of 295.4 ± 37.9 µm (Table 1). These values align well with prior studies that have reported particle sizes in similar controlled-release applications using PCL and Eudragit L100 polymers [17, 18]. The narrow particle size distribution (Span = 1.38) (Table 1) indicates high uniformity, as further evidenced in Fig. 4c. This uniformity is crucial for reproducibility in drug release profiles, as particle size distribution directly affects the pharmacokinetics of the encapsulated drug. Challenges in

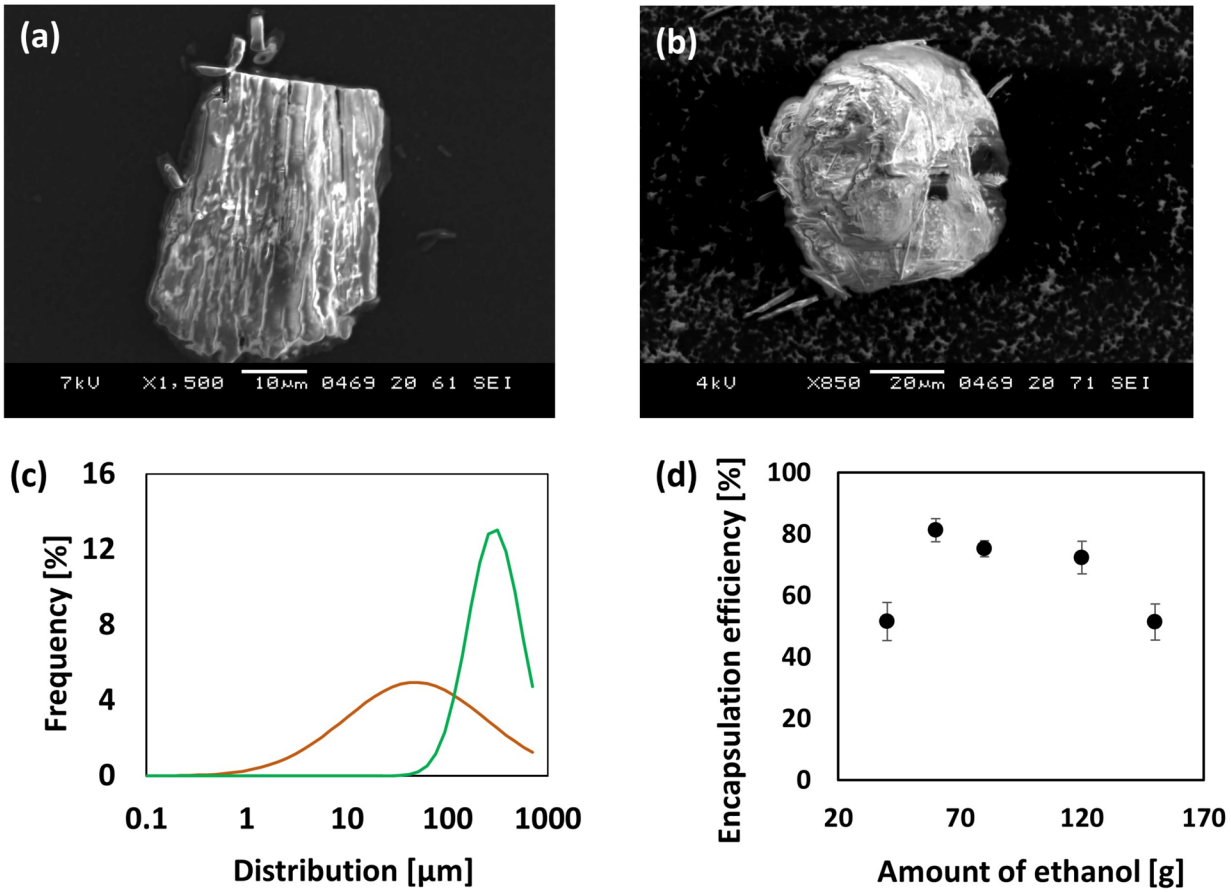

Fig. 4 SEM images of (**a**) LVF "as received"; **b** LVF-PCL microcapsules produced by PGSS process of $scCO_2$ solutions at 60 °C and 9 MPa. **c** Particle size distribution of the LVF "as received" (—), and LVF-PCL (—) produced by PGSS process of $scCO_2$ solutions at 60 °C and 9MPa. (**d**) Effects of amount of co-solvent (ethanol) on single coated LVF-GT microparticles encapsulation efficiency (%) obtained from the PGSS process using $scCO_2$ solutions at 80 °C and 9 MPa

achieving such narrow distributions during scale-up have been noted in previous research using the PGSS process, underscoring the significance of this result [19].

The encapsulation efficiency (EE) of 81.2% obtained using ethanol as a co-solvent reflects the importance of process optimization. Ethanol likely reduces the viscosity of the polymer solution, enhancing the dispersion of CO_2 and facilitating drug encapsulation. This finding is consistent with reports suggesting that ethanol can improve polymer solubility, promoting better drug-polymer interactions and higher EE [17, 21, 22]. However, increasing the ethanol concentration beyond a certain threshold (e.g., 60 mL) resulted in decreased EE, likely due to over-dilution of the polymer matrix. This trend agrees with Kalani M. and Yunus R. (2011), who found that excessive solvent can reduce the viscosity to the extent that encapsulation becomes inefficient [24]. Despite these promising results, SEM analysis of single-coated microparticles (Fig. 4b) revealed surface cracks and pores, which can lead to rapid drug release and leakage. These structural defects are commonly observed in single-coating PGSS processes and are associated with inadequate interaction between the coating material and the core drug [17]. Enhancing the coating process or introducing multiple layers has been shown to mitigate such defects [25].

To address the limitations of the single-coating system, a novel double-coating technique using glyceryl tristearate (GT) as the first layer and trimyristin (TM) as the second layer was introduced. This method significantly improved particle uniformity and surface morphology. Figure 5 shows the SEM images of LVF-GT single-coated (Fig. 5a) and GT-TM double-coated LVF microparticles (Fig. 5b) obtained from the PGSS process using $scCO_2$ solutions. The double-coated microparticles had an average size of 95.3 ± 16.5 µm, with a broader span (2.43, Table 1) compared to the single-coating process. However, the reduction in surface porosity (Fig. 5b) highlights the effectiveness of the double-coating system in producing more stable and controlled-release particles. This improvement is attributed

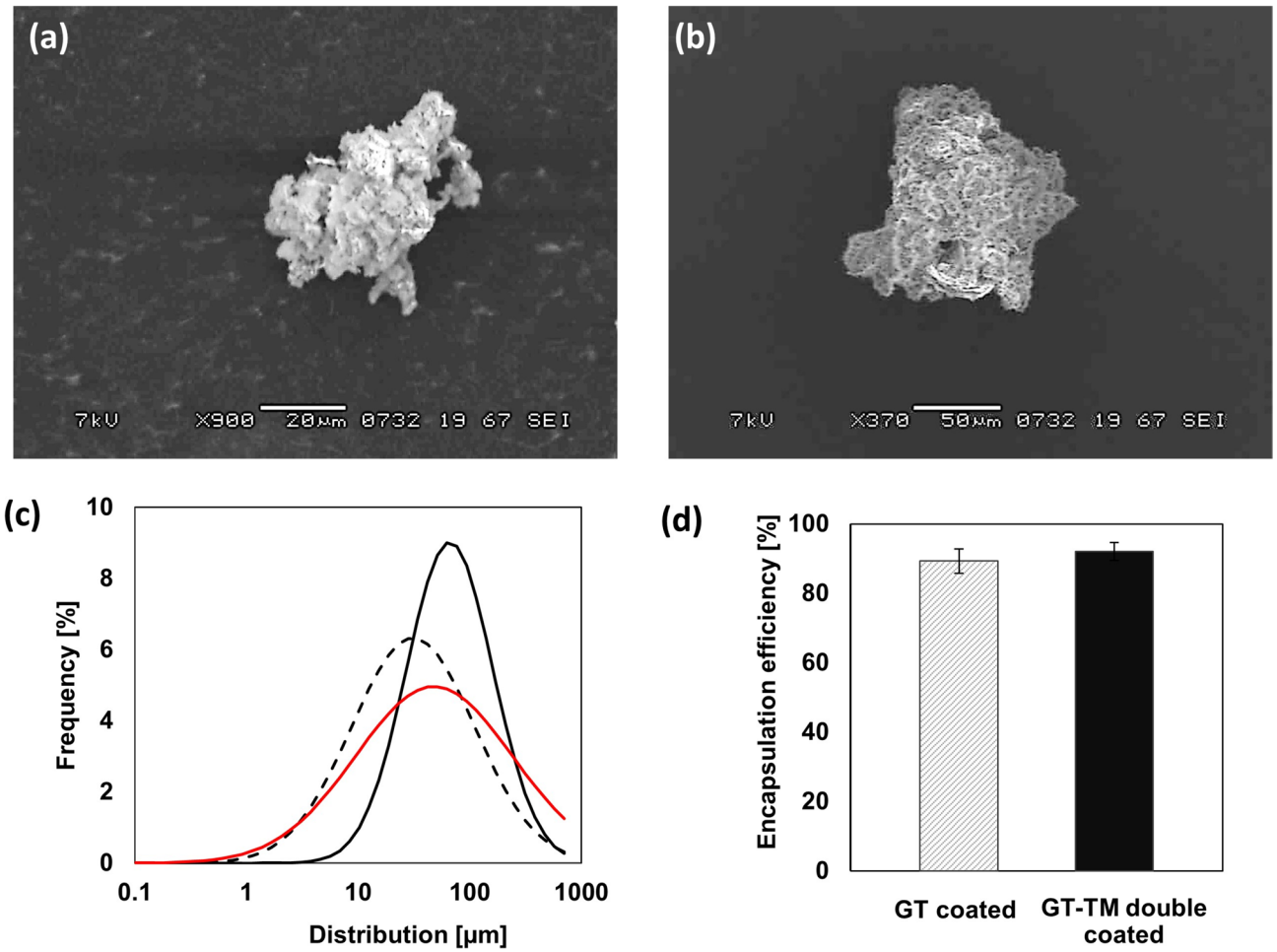

Fig. 5 SEM images of (**a**) LVF-GT single coated and (**b**) GT-TM double coated LVF microparticles obtained from the PGSS process using $scCO_2$ solutions. The initial coating was conducted with GT at a temperature of 80 °C, followed by a second coating with TM at 50 °C, both at a pressure of 9 MPa. **c** Particle size distribution of LVF "as received" (—), the TM "as received"(–), and GT-TM double coated LVF microparticles (—) produced by PGSS process of $scCO_2$ solutions at 50 °C and 9 MPa. **d** Encapsulation efficiency (%) of single coated LVF-GT microparticles and double coated GT-TM microparticles obtained from the PGSS process using $scCO_2$ solutions at 60 °C and 9 MPa

to the melting pressure depression as well as viscosity reduction of TM in ethanol, which facilitated better polymer dispersion during the secondary coating process.

The thickness of the coatings provides further insights into the success of the double-coating process (Table 2). For the single-coated particles, the thickness of the PCL coating ranged from 81.28 µm when the drug-to-polymer ratio in the single-coated system (LVF: PCL) was 1:10, while the double-coated system achieved a drug-to-excipient ratio of 1:27 but a theoretical coating thickness of 31.9 µm. Despite the larger mass of TM (GT-to-TM ratio, 1:5) in the double-coating system, the encapsulation efficiency remained high in both the single (89.3%) and double-coating (92.1%). The high encapsulation efficiency can be explained by the mechanism of encapsulation during the first coating process (Fig. 1). In the first coating (80 °C), the active ingredient is co-precipitated within the polymer matrix upon depressurization, leading to its entrapment within the core and the polymer layer of the single-coated microparticles. This encapsulation step ensures efficient retention and protection of the drug. During the second coating process, the system temperature was set at 50 °C which was lower than the melting temperature of the primary polymer. Therefore, the active ingredient remains confined to the core and the first polymer matrix. The second coating layer functions as an external protective barrier, enhancing the structural integrity and modulating the drug release profile, without contributing additional active ingredient. This is an important consideration, as studies have shown that increasing the number of coating layers can sometimes lead to a reduction in encapsulation efficiency due to increased complexity in the system [17].

3.2 FT-IR analysis

Evaluation of the presence of LVF in GT-TM double-coated microparticles and structural analysis of GT and TM before and after encapsulation were done using FT-IR, and the corresponding results are shown in Fig. 6. The major peaks in LVF at 1617 cm^{-1} and 1003 cm^{-1} were identified as arising from the stretching vibration of the aromatic C = C and p–C–F stretch, respectively. After encapsulation, 1617 cm^{-1} and 1003 cm^{-1} band, which were not observed in pure TM, appeared in the spectrum of GT-TM double-coated LVF microparticles, confirm the existence of LVF in the PGSS particles. Additionally, TM exhibits a prominent peak in the C–H stretching vibration assigned to the CH_3 group at 2913 cm^{-1}, which is also evident in the spectrum of GT-TM double-coated LVF microparticles at the same position. Furthermore, there was no shift of the absorbance observed after microencapsulation, suggesting successful encapsulation of LVF without any drug interaction within the polymer. From these results, we confirmed that LVF was well encapsulated in the GT-TM double-coated microparticles. The preservation of the characteristic peaks of LVF in the spectrum of the double-coated microparticles further validates the stability of the encapsulated drug and the integrity of the encapsulation process.

3.3 X-ray diffraction (XRD)

The XRD analysis, shown in Fig. 7, illustrates the crystalline nature of the pure drug and lipids, as well as how encapsulation affects these structures. The XRD pattern of pure LVF (Fig. 7a) exhibits sharp peaks at 2θ values of 10°, 13.66°, 19.84°, and 26.78°, indicating its crystalline form. Glyceryl tristearate (GT) and trimyristin (TM) also show distinct crystalline peaks, with GT displaying peaks at 19.8° and 23.44° (Fig. 7b), and TM at 12.96°, 19.8°, 23.64°, and 24.68° (Fig. 7d). After encapsulation using the PGSS process, the XRD pattern of the dual-layer coated LVF (Fig. 7e) shows a significant reduction in the characteristic levofloxacin peaks, suggesting that the drug is effectively encapsulated and may exist in an amorphous or

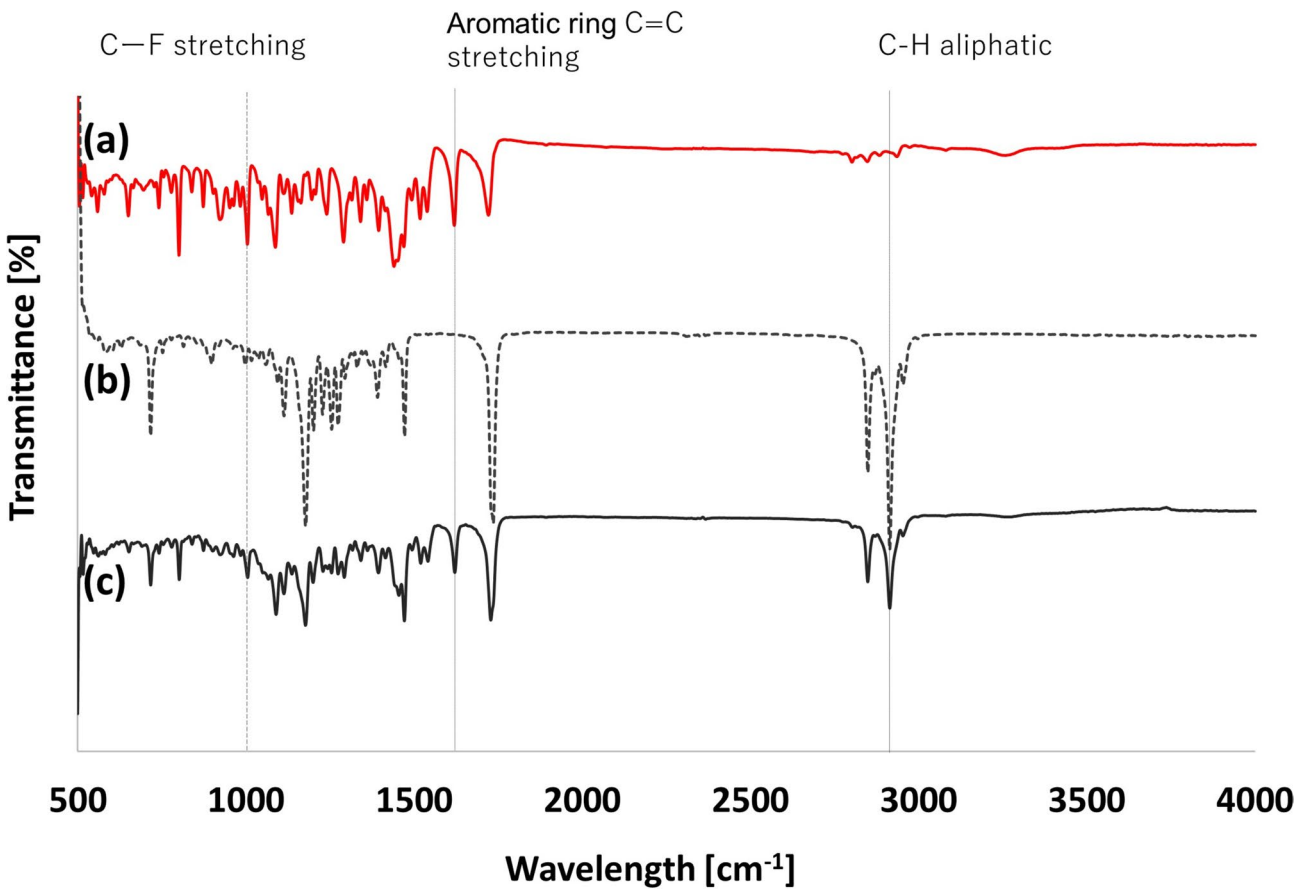

Fig. 6 FTIR spectra of (**a**) LVF "as received", (**b**) TM "as received", and (**c**) GT-TM double coated LVF microparticles produced by the PGSS process using $scCO_2$ solutions at 60 ℃ and 9 MPa

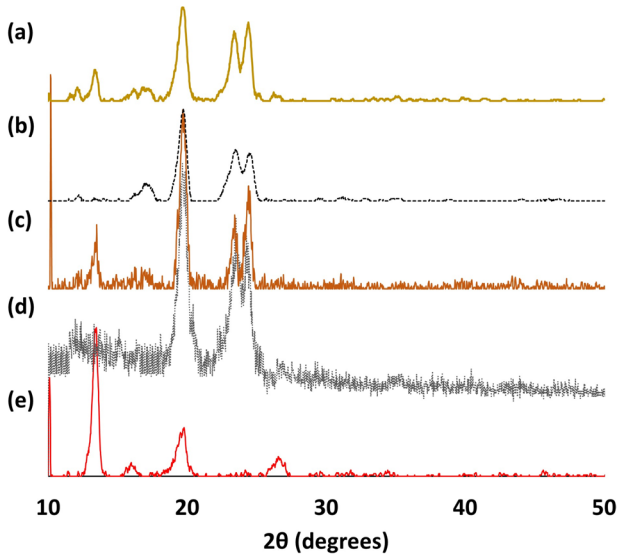

Fig. 7 X-ray diffraction (XRD) patterns showing the crystalline structure of (**a**) LVF "as received"; (**b**) GT "as received"; (**c**) single coated GT-LVF; (**d**) TM "as received"; and (**e**) GT-TM double coated LVF microparticles

molecularly dispersed state. The presence of GT and TM peaks in the final formulation confirms that both lipid coatings remain intact, providing a stable matrix for controlled drug release. The absence of additional peaks in the XRD pattern of the encapsulated microparticles suggests that no new crystalline phases were formed. This indicates that GT and TM do not chemically or structurally interact to form new compounds or solid phases.

3.4 In-vitro release profile for double-coating PGSS system

The in vitro drug release studies were conducted using vertical Franz-type diffusion cells with a dissolution medium at pH 6.8 and 37 °C over 30 days. The release profiles of LVF from single-coated LVF-PCL (1:10), LVF-GT (1:4), or LVF-GT (1:8) microparticles, as well as double-coated LVF microparticles (1:27), are presented in Fig. 8. Regardless of the drug-to-polymer ratio, both systems exhibit high encapsulation efficiency (Table 1), however, the differing release rates provide insight into the localization of the active ingredient and the impact of the coating processes. Single-coated LVF-PCL or LVF-GT microparticles with a drug-to-polymer ratio of 1:10 or 1:4, respectively exhibited an initial burst release, with approximately 80% of the loaded LVF released within the first few days, followed by a slightly accelerated release over the next 25 days (Fig. 8). In contrast, the double-coated LVF microparticles demonstrated a prolonged, zero-order release profile. The initial burst effect was significantly reduced in the double-coated microparticles, decreasing from approximately 80% to 8.5% (n = 3) on day five, and releasing only 36.2% of the loaded drug over the subsequent 25 days.

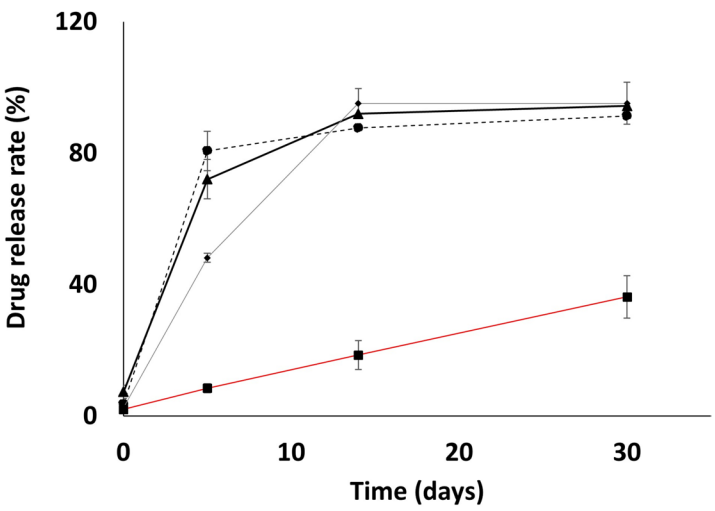

Fig. 8 Sustained release profiles of LVF from single-coated LVF-PCL 1:4 (●) or LVF-GT 1:4 (▲) or LVF-GT 1:8 (♦) microparticles and double-coated LVF microparticles (■) produced by the PGSS process using $scCO_2$ solutions. Error bars are presented as means ± SEM (n = 3). Error bars may not be visible for certain data points due to minimal variation, where SEM < 0.05

Regarding the single-coated drugs, SEM images (Fig. 4b) revealed the emergence of surface cracks on microcapsules derived from either LVF-PCL or LVF-GT, primarily triggering the initial burst release. This phenomenon was effectively mitigated by the double-coating approach, particularly with a drug-to-polymer ratio of 1:27. While cracks were also observed in the secondary coating, their lack of continuity prevented premature drug release. These findings indicate that LVF can be released slowly over an extended period, and the initial burst effect can be controlled using the double-coating PGSS process, leveraging the complementary properties of the coating materials GT and TM.

The 30 day release study, designed to evaluate the safety and stability of the microparticulate system under simulated conditions, confirmed its robustness and long-term functionality. The results of this study highlight the novelty of the double-coating PGSS system, which effectively addresses the limitations of single-coating methods by significantly reducing the initial burst release and providing a more sustained, controlled drug release profile. The use of a lipid-based double-coating system also provides additional benefits, as lipids like GT and TM have been shown to stabilize drug-loaded particles and provide controlled release properties, which is essential for sustained drug delivery applications [26, 27].

This innovative double-coating technique presents a substantial advancement in the field of controlled drug delivery, offering a more reliable and efficient method for prolonging drug release while minimizing premature release.

4 Conclusion

The PGSS process successfully produced double-coated microparticles using GT and TM as primary and secondary coatings, effectively mitigating the initial burst effect of LVF. With the addition of ethanol, the encapsulation efficiency reached 92.1%, with particles averaging 95.3 ± 16.5 µm in size at 60 °C and 9 MPa. In vitro release studies demonstrated a controlled and prolonged release of LVF over 30 days, suggesting potential benefits for reducing administration frequency and improving dosing efficiency. This study underscores the significant potential of the double-coating PGSS process for advancing drug delivery systems.

Despite these promising outcomes, further in vivo evaluation is essential to confirm therapeutic efficacy. Future work could focus on refining coating materials, enhancing particle size uniformity, and expanding the method to other drug types. Scaling up the process for industrial applications is another crucial consideration. Nonetheless, this double-coating PGSS system shows great promise for controlled drug delivery, particularly in chronic conditions requiring sustained release. It could enhance therapeutic outcomes, reduce dosing frequency, and be adapted for broader applications, including nutraceuticals and cosmetics.

Author contributions TS: write up, Data curation, formal analysis,investigation, TT: editing, MO: editing, KY: resources, HZ: resources, AS: resources, ZIS: resources, KI: editing, Validation, TS: editing, resources, Validation, AN: resources, TM: resources, YM: resources, ST: resources, KM: concept, methodology, Validation.

Funding This work was supported by the Grants-in-Aid for Scientific Research (KAKENHI) program (grant number 23K04482 and 21K04758).

Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publications Not applicable.

Competing interests The authors declare no competing interests.

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References

1. Hurst M, Lamb H, Scott L, Figgitt D. Levofloxacin. Drugs. 2012;62:2127–67. https://doi.org/10.2165/00003495-200262140-00013.
2. Deshpande D, Pasipanodya J, Mpagama S, Bendet P, Srivastava S, Koeuth T, Lee P, Bhavnani S, Ambrose P, Thwaites G, Heysell S, Gumbo T. Levofloxacin pharmacokinetics/pharmacodynamics, dosing, susceptibility breakpoints, and artificial intelligence in the treatment of multidrug-resistant tuberculosis. Clin Infect Dis. 2018. https://doi.org/10.1093/cid/ciy611.
3. Czock D, Hüsig-Linde C, Langhoff A, Schöpke T, Hafer C, De Groot K, Swoboda S, Kuse E, Haller H, Fliser D, Keller F, Kielstein J. Pharmacokinetics of moxifloxacin and levofloxacin in intensive care unit patients who have acute renal failure and undergo extended daily dialysis. Clin J Am Soc Nephrol. 2006;1(6):1263–8. https://doi.org/10.2215/CJN.01840506.
4. Ungkitwattanakul D, Chaijamorn W, Charoensareerat T, Charntrakarn P, Khamkampud O, Rattanaponpasert N, Srisawat N, Pattharachayakul S. Optimal levofloxacin dosing regimens in critically ill patients with acute kidney injury receiving continuous renal replacement therapy. J Crit Care. 2020. https://doi.org/10.1016/j.jcrc.2020.09.018.
5. Paiva JA, Pereira JM. Fluoroquinolones: another line in the long list of their collateral damage record. Crit Care Med. 2015;43(3):708–10.
6. Adepu S, Ramakrishna S. Controlled drug delivery systems: current status and future directions. Molecules. 2021;26(19):5905. https://doi.org/10.3390/molecules26195905.
7. Heng PW. Controlled release drug delivery systems. Pharm Dev Technol. 2018;23(9):833. https://doi.org/10.1080/10837450.2018.1534376.
8. Ezike TC, Okpala US, Onoja UL, et al. Advances in drug delivery systems, challenges and future directions. Heliyon. 2023. https://doi.org/10.1016/j.heliyon.2023.e17488.
9. López-López M, Fernández-Delgado A, Moyá ML, Blanco-Arévalo D, Carrera C, de la Haba RR, Ventosa A, Bernal E, López-Cornejo P. Optimized preparation of levofloxacin loaded polymeric nanoparticles. Pharmaceutics. 2019;11(2):57. https://doi.org/10.3390/pharmaceutics11020057.
10. Ziaee A, Albadarin AB, Padrela L, Femmer T, O'Reilly EJ, Walker G. Spray drying of pharmaceuticals and biopharmaceuticals: Critical parameters and experimental process optimization approaches. Eur J Pharm Sci. 2019;127:300–18. https://doi.org/10.1016/j.ejps.2018.10.026.
11. Cheow W, Hadinoto K. Enhancing encapsulation efficiency of highly water-soluble antibiotic in poly(lactic-co-glycolic acid) nanoparticles: modifications of standard nanoparticle preparation methods. Colloids Surf, A. 2010;370:79–86. https://doi.org/10.1016/j.colsurfa.2010.08.050.
12. Antonov EN, Krotova LI, Mishakov GV, Popov VK. Micronization of levofloxacine using the RESS method. Russ J Phys Chem B. 2020;14:1225–8.
13. Antonov EN, Andreevskaya SN, Bocharova IV, Bogorodsky SE, Krotova LI, Larionova EE, Mariyanats AO, Mishakov GV, Smirnova TG, Chernousova LN, Popov VK. PLGA carriers for controlled release of levofloxacin in anti-tuberculosis therapy. Pharmaceutics. 2022;14(6):1275. https://doi.org/10.3390/pharmaceutics14061275.
14. Mishima K. Biodegradable particle formation for drug and gene delivery using supercritical fluid and dense gas. Adv Drug Deliv Rev. 2008;60:411–32. https://doi.org/10.1016/j.addr.2007.02.003.
15. Mishima K, Honjo M, Sharmin T, Ito S, Kawakami R, Kato T, Misumi M, Suetsugu T, Orii H, Kawano H, Irie K, Sano K, Mishima K, Harada T, Ouchi M. Gas-saturated solution process to obtain microcomposite particles of alpha lipoic acid/hydrogenated colza oil in supercritical carbon dioxide. Pharm Dev Technol. 2016;21:737–48. https://doi.org/10.3109/10837450.2015.1049707.
16. Ono K, Sakai H, Tokunaga S, Sharmin T, Aida TM, Mishima K. Encapsulation of lactoferrin for sustained release using particles from gas-saturated solutions. Processes. 2021;9(1):973. https://doi.org/10.3390/pr9010073.
17. Tokunaga S, Ono K, Ito S, Sharmin T, Kato T, Irie K, Mishima K, Satho T, Harada T, Aida TM, Mishima K. Microencapsulation of drug with enteric polymer Eudragit L100 for controlled release using the particles from gas saturated solutions (PGSS) process. J Supercrit Fluids. 2021;167: 105044.
18. Sharmin T, Tashiro H, Sakai H, Shibata K, Ema K, Irie K, Satho T, Mishima K. Microencapsulation of pyriproxyfen using the particles from gas-saturated solutions process as a controlled-release system. Asia-Pac J Chem Eng. 2024. https://doi.org/10.1002/apj.3043.
19. Varona S, Martin A, Cocero MJ, Duarte CMM. Encapsulation of Lavandin essential oil in poly-(ε-caprolactone) by PGSS process. Chem Eng Technol. 2013;36:1187–92. https://doi.org/10.1002/ceat.201200592.
20. Liu F, Lizio R, Meier C, Petereit H-U, Blakey P, Basit AW. A novel concept in enteric coating: a double-coating system providing rapid drug release in the proximal small intestine. J Control Release. 2009;133:119–24.
21. Rojo S, Martín Á, Calvo E, Cocero M. Solubility of polycaprolactone in supercritical carbon dioxide with ethanol as cosolvent. J Chem Eng Data. 2009;54:962–5. https://doi.org/10.1021/JE8007364.
22. Tsivintzelis I, Pavlidou E, Panayiotou C. Biodegradable polymer foams prepared with supercritical CO_2–ethanol mixtures as blowing agents. J Supercrit Fluids. 2007;42(3):265–72. https://doi.org/10.1016/j.supflu.2007.01.001.
23. King JA, Ratner BD. Levofloxacin impregnation and extended release: Concentration model for insulin catheters. Biomed Eng Adv. 2023;5: 100081. https://doi.org/10.1016/j.bea.2023.100081.
24. Kalani M, Yunus R. Application of supercritical antisolvent method in drug encapsulation: a review. Int J Nanomed. 2011;6:1429–42. https://doi.org/10.2147/IJN.S19021.
25. Saurer E, Jewell C, Kuchenreuther J, Lynn D. Assembly of erodible, DNA-containing thin films on the surfaces of polymer microparticles: toward a layer-by-layer approach to the delivery of DNA to antigen-presenting cells. Acta Biomater. 2009;5(3):913–24. https://doi.org/10.1016/j.actbio.2008.08.022.

26. Kulkarni C, Moinuddin Z, Patil-Sen Y, Littlefield R, Hood M. Lipid-hydrogel films for sustained drug release. Int J Pharm. 2015;479(2):416–21. https://doi.org/10.1016/j.ijpharm.2015.01.013.
27. Cai X, Fan B, Thang S, Drummond C, Tran N, Zhai J. Paclitaxel-loaded cubosome lipid nanocarriers stabilized with pH and hydrogen peroxide-responsive steric stabilizers as drug delivery vehicles. J Mater Chem B. 2023;11(2):403–14. https://doi.org/10.1039/d2tb01530g.

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