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Original Article

Synthesis and Evaluation of a Polycaprolactone-methoxy-Polyethyleneglycol Copolymer Nano-system for Curcumin and Tacrolimus Release

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Abstract

Conventional drug delivery methods are not highly efficient due to low bioavailability, poor absorption, and inadequate retention time of some medications. In addition, to achieve the appropriate therapeutic effect, a higher dosage of drugs might be administrated, which may result in undesired side effects. In order to overcome these challenges, utilizing drug delivery systems such as micelles could be beneficial. Micelles are amphiphilic compounds with a hydrophilic shell and a hydrophobic core. Micelles could encapsulate hydrophobic drugs such as Curcumin and tacrolimus, anti-inflammatory and immunosuppressive agents, respectively, and improve their bioavailability. This study synthesized a Methoxy-Polyethyleneglycol-Polycaprolactone biodegradable nano-system by precipitation method for curcumin and tacrolimus encapsulation. Afterward, micelles were characterized for size, morphology, drug loading capacity, and drug release. As a result, the average molecular weight of the copolymer was 36744 g/mol. The nano-system size was analyzed using Dynamic light scattering and Atomic force microscopy tests, resulting in 137.6 nm and 37.9 nm, respectively. In addition, the drug loading efficiency was 42.06%, and after 96 hours, about 0.7mg of Curcumin was released while no Tacrolimus was detected. Since Tacrolimus is retained in the system, we can conclude that this system is not suitable for the simultaneous release of two drugs.

Keywords: Nanosystem; Micelles; Caprolactone; Polyethylene glycol; Curcumin; Tacrolimus; Drug delivery.

1. Introduction

Curcumin and tacrolimus are two potent drugs with potential therapeutic effects that can be used for preventing and controlling various diseases. Curcumin ($C_{21}H_{20}O_6$), a natural polyphenol found in turmeric, exhibits anti-inflammatory, anti-proliferative, antibacterial, and antioxidant properties. With these properties, curcumin could be effective in treating diseases such as atherosclerosis, diabetes, and arthritis and accelerating the wound-healing process. However, its clinical usage is limited due to its poor aqueous

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solubility, rapid metabolism, and poor bioavailability [1-3]. Tacrolimus, an immunosuppressive drug obtained from the fungus *Streptomyces tsukubaensis*, is also limited because of its hydrophobic nature, narrow therapeutic index, and risk of drug interactions [4, 5].

In order to overcome these challenges, utilizing different drug delivery systems, such as micelles, could be beneficial. Polymeric micelles are core/shell structures of amphiphilic block copolymers [6]. With their hydrophobic core and hydrophilic shell, they can enhance the permeability and bioavailability of lipophilic drugs such as curcumin and tacrolimus, resulting in a more efficient treatment process and reduction in drug side effects [7, 8]. Micelles are classified as nano-carriers, ranging from 10 to 100 nm in diameter; therefore, they have a high permeability and are suitable for targeted drug delivery. They can self-assemble in a proper solvent and above critical micelle concentrations. Thus, their preparation method is easy and cheap. In addition, their sterilization process is simple, making them a great choice for biomedical applications [9, 10].

Common hydrophobic polymers for micelle production are Polypropylene oxide (PPO), Polylactic acid (PLA), and Polycaprolactone (PCL). In addition, for hydrophilic shells, Polyethyleneglycol (PEG) is mostly utilized [11]. PCL is a semi-crystalline aliphatic polyester, which is biocompatible and biodegradable. It possesses proper viscoelastic properties and is suitable for producing different structures. It can also easily blend with other polymers, thus ideal for copolymer production [12]. PEG is a non-toxic, inert polyether highly soluble in water and organic solvents. In addition, it can initiate the ring-opening polymerization for ε-caprolactone (CL) in the presence of a catalyst and form PEG-PCL copolymers suitable for micelle production [13-15].

In this research, we used an mPEG (methoxy-polyethylene glycol) polymer, a PEG chain with a methyl functional group, to synthesize an mPEG-PCL copolymer. Afterward, this copolymer was employed for producing Curcumin and tacrolimus-loaded micelles by nano-precipitation method. Finally, the drug's solubility and controlled release properties were investigated for this system.

2. Materials and Methods

2.1. Materials

The materials used in this study included m-PEG (code 4-74-9004, Sigma-Aldrich) with a molecular weight of 5000 g/mol, monomer caprolactone (Ɛ-CL) (3-44-502 from Merck, Germany) with the molecular weight of 114/14 g/mol and linear formula $C_6 H_{10} O_2$, catalyst Sn(oct)2 (code 0-10-301, Sigma-Aldrich) with the molecular weight of 405/12 g/mol and linear formula of [CH3(CH2)3CH(C2H5)CO2] 2 Sn, diethyl ether (code 6-10-115, Sigma-Aldrich), chloroform (code 3-66-67, Merck, Germany), ethanol 96% (Bidistan, Tween 20, code 5-64-9005m Apllichem). Potassium chloride (KCL, code 7-40-7447, ACROS), sodium chloride (NaCl, code 5200, Qatran Shimi), potassium phosphate (H2KPO4, code 0-77-7778, ACROS, and disodium hydrogen phosphate (Na2HPO4, code 4-79-7558, Scharlau) were the other procured materials. Tacrolimus and Curcumin were prepared as 1 mg capsules from the Zahrawi Company.

2.2. Copolymer Synthesis

Micelles were synthesized according to Danafar et al. [16]. First, 4 g of caprolactone, 2 g of mPEG, and 0.01 mmol of Sn(Oct)2 were heated to 120℃ in a flask in an oil bath for 11 h. Accordingly, the synthesis of the mPEG-PCL copolymer was initiated by the ring-opening polymerization process of caprolactone. After this process, the obtained material was cooled at room temperature for ten hours and turned into a solid state. Then, the obtained copolymer was dissolved in chloroform, and diethyl ether was added to the solution to precipitate the polymer. Finally, the copolymer was placed in a rotary evaporator at room temperature for 24 hours to dry the polymer completely after removing the chloroform and diethyl ether from the system. After the copolymer synthesis, the copolymer formation was confirmed using various analyses, such as proton nuclear Magnetic resonance spectroscopy (1H NMR) and Fourier transform infrared spectroscopy (FT-IR), to identify the copolymer chemical structure and Gel permeation chromatography (GPC) to determine the average molecular weight and distribution of mPEG-PCL copolymers.

2.3. FTIR Analysis

The ester bond between PCL and mPEG was identified using FTIR by placing the substance in an FTIR apparatus (Terkinelmer FRONTIER, USA), and the results were analyzed in the wavelength range of 500-4000 cm^{-1}.

2.4. NMR Analysis

Due to the less accurate results of FTIR, the structure and composition of the -(CH2)3, -OCCH2-, CH2COO copolymer should also be determined using the NMR analysis. For this purpose, 2 mg of the copolymer was dissolved in deuterated chloroform (CDCL3) and then studied at 400 MHz using a BRUKER BR X500 AVANCE H1NMR device.

2.5. GPC Analysis

For the GPC analysis, tetrahydrofuran (THF) was determined as the mobile phase with a rate of 0.5 ml/min and a 0.2-1 mg/ml concentration. The molecular weight of the copolymer was determined by analyzing the sample in an HPLC9100YL chromatography column.

2.6. Synthesis of drug-containing micelles

Drug-containing micelles were prepared by the nanoprecipitation method. To this end, 8 mg of Curcumin, 8 mg of tacrolimus, and 30 mg of the copolymer were dissolved in 2 cc of ethanol at 60℃. The solution was evaporated in a rotator at 55℃ at 70 rpm. Next, 25 mg of heated distilled water was slowly added to the mixture to form the micelles. Finally, the solution was centrifuged at 12,000 rpm for 45 min to separate the drug and the polymer. The sample was freeze-dried for 48 hours to remove the residual solvent.

2.7. Micelle Size

The micelle size distribution was determined by dynamic light scattering (DLS). For this purpose, 0.1 mg of the sample was dissolved in deionized water and analyzed in a Malvern Nano ZS Zetasizer system.

2.8. Morphology of Particles

The morphology of particles was examined using an Atomic force microscopy (AFM) device by dissolving 0.1 mg of the produced micelle in double-distilled water. Next, the obtained solution was diluted and placed on a silicon layer. After drying, the sample was placed in an AFM device (DualSGPe C-26, Denmark) and imaged in 3 μm × 3 μm dimensions.

2.9. Micelle Loading Capacity

The Drug loading capacity (DLC) and Encapsulation efficiency (EE) were determined using ultraviolet–visible spectroscopy (UV-Vis). First, 1 mg of Tacrolimus was dissolved in 1 mL of ethanol, and the absorbance rate was examined in different solution dilutions at 200-600 nm wavelengths. Similarly, 0.5 mg of Curcumin was dissolved in ethanol, and its absorbance rate was studied in the mentioned conditions. Finally, based on the data, a standard concentration diagram was obtained according to the drug absorption. Then, a given amount of drug-containing micelles was dissolved in ethanol, and the absorbance was determined at 430 and 212 nm wavelengths for Curcumin and tacrolimus, respectively. Based on the obtained data, DLC and EE values were obtained using Eqs. (1) and (2) [17].

DLC = (drug weight in micelles)/(micelle weight) × 100% (Eq. 1),

EE = (drug weight in micelles)/(drug initial weight) × 100% (Eq. 2).

2.10. Stability of Micelles

The stability of micelles was evaluated by placing the sample in phosphate-buffered saline (PBS) (pH = 7.4) at room temperature. In addition, its stability was then evaluated by DLS at 5-day intervals up to 25 days.

2.11. Drug Release Profile

Drug release was evaluated by applying the dialysis method. To this end, 10 mg of dried drug-containing micelles was dissolved in 4 ml of PBS. The solution was placed in a dialysis bag (Mw 12Kda) at 37℃ in 45 ml of PBS. At predetermined times, 2 mL of the dialysis medium was replaced with 2 mL of fresh PBS. Finally, the concentration of the drug released in a known period was measured by UV-visible

3. Results and Discussion

3.1. Synthesis of the mPEG-PCL Copolymer

The hydroxyl group in the mPEG started the ring-opening polymerization process for caprolactone, followed by the synthesis of the mPEG-PCL copolymer (**Figure 1**) [18].

3.2. FTIR Analysis

Absorbance values for the carbonyl group in ester (C=O) are 1730-1750 cm^{-1}. The ether group (C-O) has two or more bands in the 1000-1400 cm^{-1} [19]. According to the result obtained from the FTIR test, several bands can be observed in the range of 1000-1400 cm^{-1}, indexed to the C-O group. At the 1727 cm^{-1} point, a sharp peak confirms the presence of the C=O group.

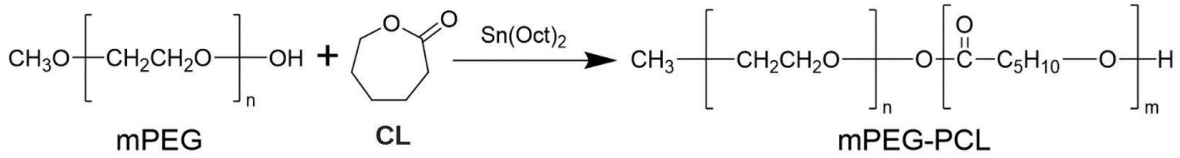

Figure 1. Ring-opening polymerization of CL by hydroxyl functional group in mPEG, in the presence of Sn(Oct)2 catalyst and formation of mPEG-PCL copolymer.

A slight difference between the obtained number and the determined interval may result from noise or device error. In general, the FTIR results indicate the presence of an ester bond in the substance (**Figure 2**). However, this bond alone does not indicate copolymer formation because it can also be observed in caprolactone.

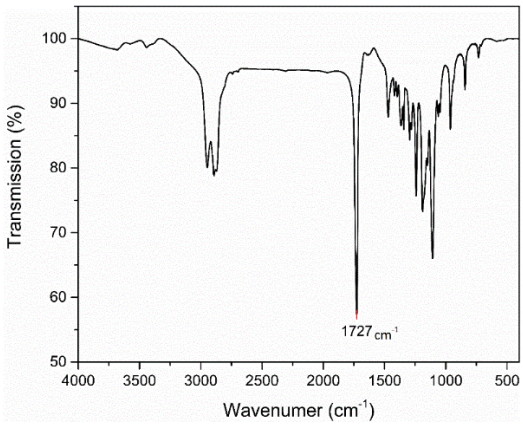

Figure 2. The result of the FTIR test that the wave number 1727 cm^{-1} and the range 1000-1400 cm^{-1}, respectively, indicate the presence of C=O and C-O functional groups.

3.3. NMR Analysis

In **Figure 3**, 1.36 and 1.63 ppm peaks represent methylene protons in -(CH2)3, and 2.28 and 4.04 ppm peaks characterize methylene protons for -OCCH2- and -CH2COO- in the PCL part, respectively. The strong peak at 3.62 ppm corresponds to methylene protons in -CH2CH2O- in the mPEG part. The weak peak observed at 3.38 ppm indicates the methyl protons in -CH3 in the mPEG part [20-23]. In addition, a very weak peak at 2.4 ppm indicates four protons at the end of the mPEG part, located near the -COO- group of the PCL part. Barki et al. and Gou M et al. obtained similar results in their articles [24, 25].

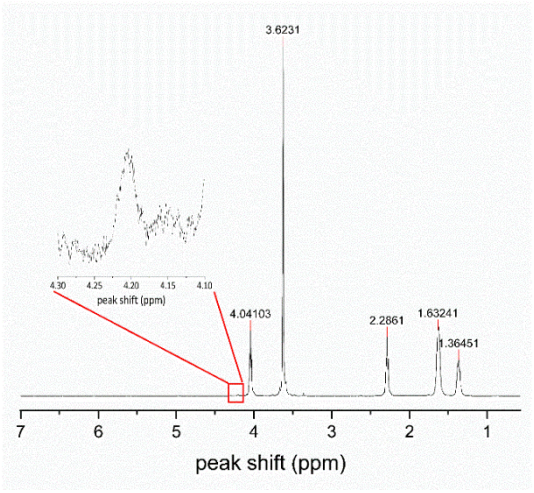

Figure 3. H1NMR diagram of mPEG-PCL copolymer. The weak peak near 2.4 ppm indicates the -CH2- group of the mPEG segment, located near the -COO- group of the PCL segment. This peak indicates the connection of two polymer parts by an ester bond.

3.4. GPC Analysis

The GPC analysis revealed the polymer's average weight and chain length of 36744 g/mol (**Figure 4**). The average molecular weight depends on various factors, such as the molecular weight of individual components or the copolymer synthesis method and conditions. Bernabeu et al. used mPEG and PCL with the same molecular weight as the present study (i.e., 5000 and 114.14 g/mol, respectively) [26]. However, they obtained a molecular weight of 29993 g/mol for the resulting copolymer due to

different synthesis conditions. FTIR, NMR, and GPC results revealed the successful synthesis of the mPEG-PCL copolymer. After ensuring the copolymer formation, micelles were produced using the nan-participation method.

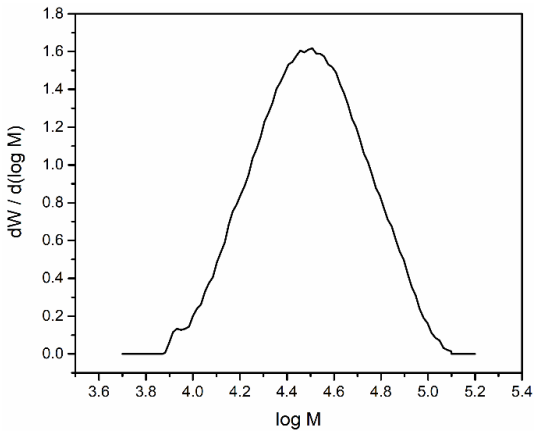

Figure 4. The molecular weight distribution of the synthesized copolymer.

3.5. Micelle Size

According to the DLS result (**Figure 5**), an average size of 137.6 nm was measured for micelles.

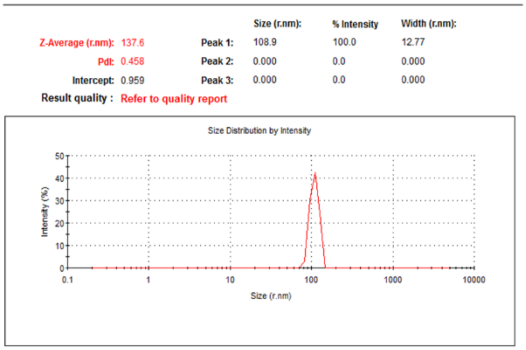

Figure 5. Average particle size distribution: micelle size 137.6 nm and PDI=0.458.

Some water was absorbed into the system because the micelles were dissolved in PBS and due to the presence of mPEG. Therefore, the obtained number is related to the size of water-coated particles and is larger than the actual particle size. Polydispersity Index (PDI) values can range from zero to one. However, numbers higher than 0.7 are highly polydispersed and unsuitable for DLS analysis [27]. PDI value for this study was 0.458, indicating that the particles were moderately polydispersed.

3.6. Particle Morphology

An average size of 37.9 nm was measured for the micelle particles using the AFM method. In this analysis, the drops are poured on the silicon surface, thereby losing their water. Therefore, they were not coated with water, and the reported number is smaller than the size obtained in DLS. **Figure 6** presents the spherical morphology of these particles, with a maximum particle size of 48.8 nm.

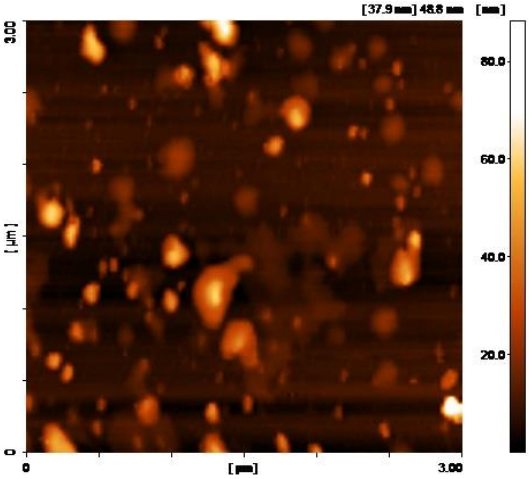

Figure 6. Nanostructure and micelles particle size: the average particle size is 37.9.

3.7. Drug Content in the Micelle and Drug Loading Efficiency in the System

The loaded drug content and the system efficiency were calculated using Eqs. (1) and (2), respectively. According to **Table 1**, the loading rate of Tacrolimus is higher than that of Curcumin, probably due to the greater hydrophobicity of this drug. Moreover, the greater tendency of Tacrolimus

to remain in the system may prevent Curcumin entry into the system and reduce its loading.

Table 1: Curcumin and tacrolimus drug loading rate in prepared nano-system.

Medicine name	Amount of drug loaded in micelles
Curcumin	5.1%
Tacrolimus	9.5%
Curcumin and tacrolimus	14.6%

The drug loading efficiency is higher for Tacrolimus (**Table 2**). An overall efficiency of 42.06% was obtained for the system when loading two drugs simultaneously. This percentage shows that the system cannot maintain the drug, and more than half of the drug is released.

Table 2. Drug loading efficiency.

Medicine name	Drug loading efficiency
Curcumin	29.3%
Tacrolimus	54.7%
Curcumin and Tacrolimus	42.06%

3.8. Stability of Micelles

The stability of micelles should be evaluated to ensure the structure integrity and the absence of accumulated particles. For this purpose, the diagram of DLS test results (**Figure 7**) was obtained at 5-day intervals for one month.

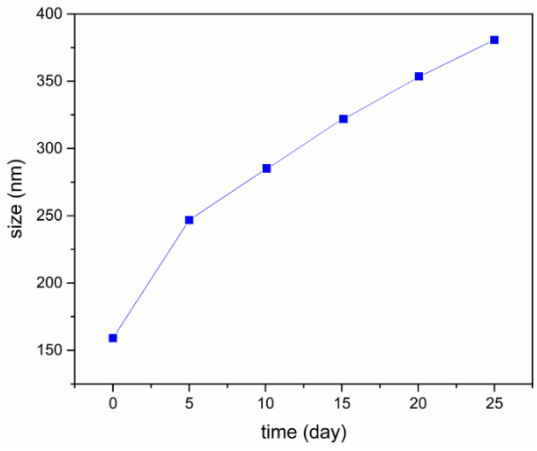

Figure 7. Increasing the size of the micelles during one month and at five days intervals.

This diagram shows an increase in the micelle size over time, which occurs due to the hydrophilic m-PEG and, consequently, water absorption by the system. This diagram has a steeper slope in the first five days, indicating the higher water absorption rate in the first days. In this respect, Zamani et al. achieved similar results on the physical stability of micelles [28].

3.9. Drug Release Profile

Drug release was studied in the PBS solution to investigate the effects of chemical and biochemical factors on the release of Curcumin and Tacrolimus from the prepared micelles. **Figure 8** presents the Curcumin release rate within 96 h. According to this figure, the drug release rate was high in the early hours and decreased over time. After about 40 h, the Curcumin release rate increased with a stable slope. Similar to a study on Curcumin release from mPEG-PCL micelles by Danafer et al., no sudden release occurred in our experiment [29].

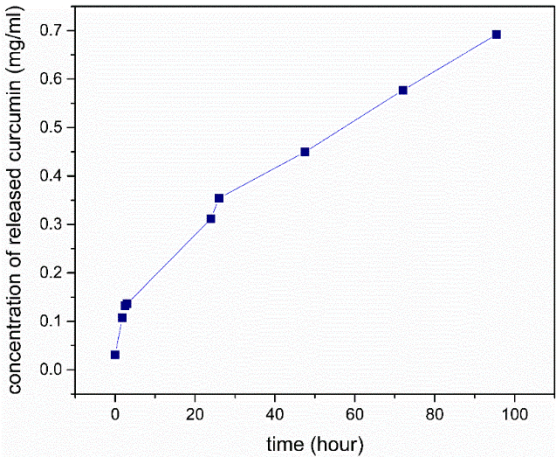

Figure 8. The release rate of curcumin from micelles in 96 hours.

Tacrolimus release was tested through its absorption value at 212 nm. According to the obtained results, absorption of 0 indicates that the drug is not released from the system. In

contrast, Wang et al. reported the suitability of the PCL-PEG-PCL system for Tacrolimus release [30]. The difference in the results might be attributed to the interaction of Tacrolimus and Curcumin in this study.

4. Conclusion

Micelles are one of the suitable systems for releasing hydrophobic drugs, which can increase drug efficiency and reduce the corresponding side effects. This system can be used for drug delivery to treat and prevent diseases such as atherosclerosis. In our study, mPEG-PCL micelles were synthesized for Curcumin and Tacrolimus encapsulation. Initially, the mPEG-PCL copolymer was synthesized by the nanoprecipitation method. Then, the functional groups, chemical structure, and molecular weight were identified using FTIR, NMR, and GPC analyses. After confirming the polymer synthesis, Tacrolimus and Curcumin were loaded into the micelles, followed by examining the size, morphology, and stability of micelles. The results revealed a spherical and homogeneous morphology of micelles with a size of 37.9 nm. The system dimensions increased over time due to the presence of hydrophilic mPEG and water absorption by micelles. The simultaneous drug loading efficiency in the system was equal to 42.06%, suggesting the drug wastage. The drug release study showed that Curcumin was released from the system; meanwhile, Tacrolimus was trapped in the system, and the release did not occur for this drug. The higher tendency of Tacrolimus to remain in the system or the effect of Curcumin on this drug might have precluded its release from the system. Overall, it can be concluded that the prepared micelles are unsuitable for the simultaneous loading of Tacrolimus and Curcumin due to a high drug loss in the system and no release of tacrolimus.

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Conflict of interest

The authors declare to have no conflict of interest.

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