The production of curcumin-loaded PLGA/PEG nanoparticle for the treatment of Alzheimer's disease

Ece GULER[1,2] (ID) **, Muhammet Emin CAM[1*]** (ID)

[1] Department of Pharmacology, Faculty of Pharmacy, Marmara University, Basıbuyuk 34854 Istanbul, Türkiye
[2] Department of Pharmacology, Faculty of Pharmacy, Istanbul Kent University, Kagıthane 34406 Istanbul, Türkiye
*   Corresponding Author. E-mail: muhammet.cam@marmara.edu.tr (M.E.C.); Tel. +09-216-77752 00.

ABSTRACT: Alzheimer's disease (AD) is the most common type of dementia in the world. This neurodegenerative disease affects 6.5 million people's lives by leading to functional impairments such as memory loss and cognitive regression. In the treatment of AD, there is no drug for radical treatment in the worldwide. Current treatment strategies provide relief to the symptoms. Therefore, curcumin (CUR) was preferred as a promising alternative therapeutic approach in this study. By using the double emulsion solvent evaporation technique, PLGA/PEG nanoparticles were produced and CUR was loaded to these nanoparticles (CNP). After that, the chemical structures and morphologies of CNP and PNP were analyzed by using FTIR and SEM. As a result, it was proven that these nanoparticles were produced by using PLGA and PEG polymers and CUR was loaded to these nanoparticles successfully.

KEYWORDS: Alzheimer's disease; curcumin; nanoparticle; PLGA; PEG

1. INTRODUCTION

Dementia is a disease characterized by the deterioration of memory and related mental abilities as well as a decline in daily cognitive functioning. Approximately 50 million people worldwide currently live with dementia. Alzheimer's disease (AD), the most common type of dementia, is a neurodegenerative disease that leads to functional impairments such as memory loss and cognitive regression [1,2]. AD is caused by the accumulation of amyloid β as plaques and hyperphosphorylated tau proteins as neurofibrillary tangles (NFTs) in brain tissue. In the 2022 report of the American Alzheimer's Association, it is known that 6.5 million individuals aged 65 and over in the United States have AD. Unfortunately, it is estimated that this number may reach 13.8 million by 2060. Additionally, the number of patients with AD is expected to reach 107 million worldwide by 2050 [3,4].

Currently, there has been no radical treatment for the treatment of AD, which seriously affects the lives of many people all around the world. The developed treatment strategies are only aimed at relieving the symptoms of the disease, slowing its progression, and improving life quality. The drugs approved by the United States Food and Drug Administration (FDA) are divided into two groups as FDA definition: acetylcholinesterase inhibitors (AChEIs) and N-methyl-D-aspartate (NMDA) receptor antagonists. ACHEIs (donepezil, rivastigmine, galantamine) are the most widely used class of drugs in the treatment of AD. These drugs have been shown to alleviate cognitive symptoms, provide a significant benefit in activities of daily life by increasing acetylcholine levels in the brain, and also treat mild to moderate AD symptoms. On the other hand, uncompetitive NMDA receptor antagonist memantine helps regulate glutamate signaling in the brain and works to treat mild or moderate AD symptoms. However, these drugs cause various gastrointestinal side effects such as diarrhea or nausea [5,6].

Curcumin (CUR) is one of the most promising and common therapeutic approaches used in the treatment of AD. The neurological benefits of CUR are relevant for AD, Parkinson's disease, brain tumors, multiple sclerosis, traumatic brain injuries, ischemia, and depression. It is believed that CUR acts against AD through multiple mechanisms. These mechanisms include inhibiting the formation of Aβ and tau hyperphosphorylation, decreasing acetylcholinesterase levels, modulating microglia, chelating metals, having antioxidant activity, regulating insulin signaling pathway, and lowering cholesterol [7-9]. However, the usage of CUR has been encountering some obstacles. It has exceptionally low water solubility, which means that it is rapidly excreted from the body and also has low oral bioavailability [10].

Nanotechnology has the potential to be effective in both detection and treatment of a wide range of diseases [11]. Drug delivery systems (DDS) produced using nanotechnology improve drug stability, controlled distribution, pharmacodynamics, and pharmacokinetic profiles [12]. The selection of the appropriate drug delivery system is crucial for AD due to the pathophysiology of the disease and the physicochemical properties of the drugs used for the treatment of AD. The advantages of nanotechnological DDS include the ability to target drugs in a tissue or organ-specific manner, the binding of two or more active agents to a nanocarrier, and the ability to monitor the efficacy of treatment [13]. Various nanosized materials, especially nanoparticles and nanofibers, are used in the development of these systems [14].

Nanoparticles provide controlled release of drugs from the matrix, thereby increasing the bioavailability of the drug and reducing the dosage frequency [15]. Moreover, they are frequently preferred due to their increased formulation efficiency by facilitating absorption to target tissues, providing stabilization for drug kinetics and distribution, allowing hydrophilic and hydrophobic drugs to be used together, and being able in combined therapy [16]. Poly(lactic-co-glycolic acid) (PLGA) is a biocompatible, biodegradable, and well-tolerated polymer widely used in nanoparticle production. In the production of drug-loaded nanoparticles, copolymerization of PLGA with polyethylene glycol (PEG) and the use of appropriate binders facilitate the crossing of drugs through the blood-brain barrier (BBB) [17].

In this study, pure PLGA/PEG nanoparticles (PNPs) were produced for developing a new DDS. Then, CUR was selected as a therapeutic agent, and loaded to these nanoparticles to provide a radical treatment for AD. The chemical structures of CUR-loaded PLGA/PEG nanoparticles (CNPs) were also elucidated by Fourier transform infrared spectroscopy (FT-IR) analysis.

2. RESULTS

2.1. Fourier transform infrared spectroscopy (FTIR)

FTIR was used to analyze the chemical structure of the nanoparticles. Both CUR and polymers used to produce the nanoparticles were analyzed individually by considering their characteristic peaks. The C-H single bond stretching peak at 2879 cm^{-1}, C=O stretching vibration at 1633 cm^{-1}, CH_2 stretching peak at 1453 cm^{-1}, C-O single bond (ether) stretching peak at 1097 cm^{-1} were observed as the characteristic peaks of PEG [18]. Also, peaks at 1460 cm^{-1} and 1340 cm^{-1} represent C-H deformation vibrations. In addition, the peaks at 1281 cm^{-1} and 1239 cm^{-1} belong to O-H bending vibrations [19]. Besides these peaks, the absorption peak at 1127 cm^{-1} is associated with the C-O-C characteristic vibration of PEG [20]. For PLGA, the broad peak at 3502 cm^{-1} shows O-H stretching vibration. The characteristic bands at 2998 and 2954 cm^{-1} are related to aliphatic C-H stretching vibrations (C-H, C-H_3, and C-H_2). The stretched -C=O groups in PLGA were observed at 1745 and 1630 cm^{-1}. The stretching of the C-O group was obtained at 1083 cm^{-1} [21,22]. The characteristic peaks of CUR were analyzed and the O-H stretching vibration belonging to the phenol group at 3232 cm^{-1}, -CH_3 stretching vibration at 2906 cm^{-1}, C-O stretching vibration at 1024 cm^{-1}, bands of C=O and C-O (ether) groups at 1625 and 1589 cm^{-1}, -C-O elongation of OH single bond group at 1500-1400 cm^{-1}, specific peaks of C-H alkene group and C-H bending vibration at 700, 806 and 960 cm^{-1} were observed [23,24]. Considering all these characteristic peaks, the peaks at 2940, 1643, 1461, 1421, 1286, and 892 cm^{-1} were found compatible with the characteristic peaks of PLGA and PEG after analyzing of PNP. Similarly, CNP spectroscopy was proven that CUR was encapsulated in CNP with peaks at 3365, 2944, 1629, 1025, 964, and 813 cm^{-1}. Thus, the presence of PEG and PLGA polymers claimed to be used in nanoparticle production, and CUR, used as a drug in these nanoparticles was proven following the analysis of this assay (Figure 1).
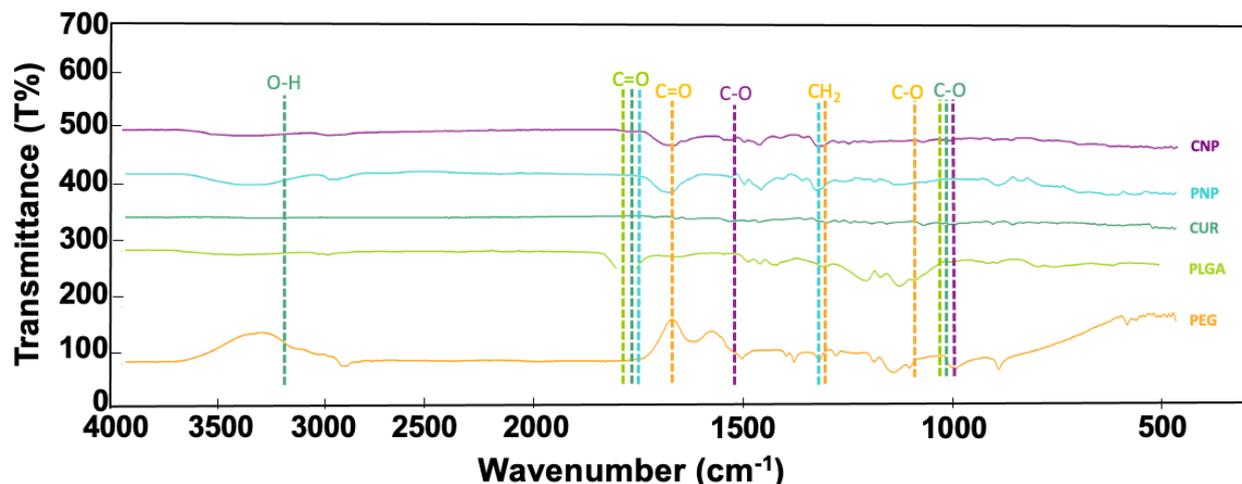
Figure 1. FTIR results of pure poly-lactic-co-glycolic acid (PLGA), pure polyethylene glycol (PEG), pure curcumin (CUR), pure PLGA/PEG nanoparticles (PNPs), CUR-loaded PLGA/PEG nanoparticles (CNPs).

2.2. Scanning electron microscopy (SEM)

The morphologies and distributions of the nanoparticles produced by the double emulsion solvent evaporation technique were analyzed by using SEM and the results were given in Figure 2. The diameter of the produced PNP was measured as 202 ± 1.28 nm. After that, the CUR was encapsulated in these nanoparticles and the particle sizes of the nanoparticles were measured again. An increase was obtained in the particle diameter from 202 ± 1.28 nm to 216 ± 1.84 nm after encapsulating CUR.
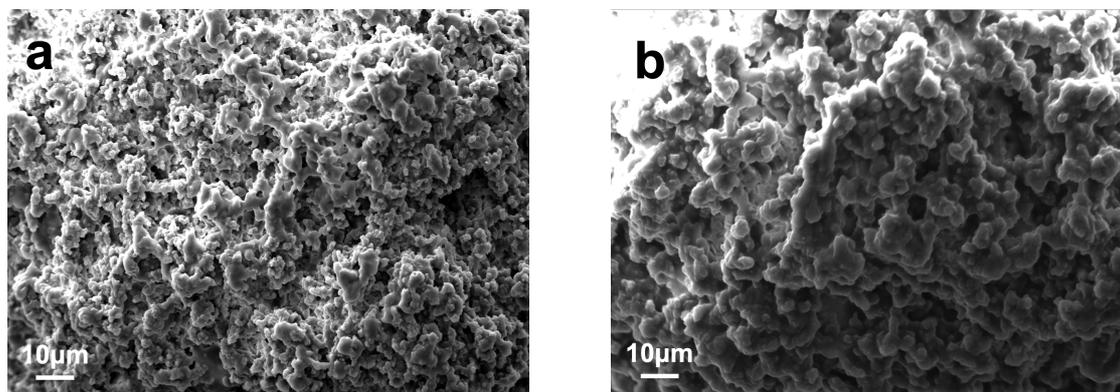

Figure 2. SEM images of nanoparticles : (a) pure PLGA/PEG nanoparticles (PNPs) and (b) CUR-loaded PLGA/PEG nanoparticles (CNPs)

3. DISCUSSION

The FTIR analysis proves whether the chemical structure and binds belong to polymers and drugs that are claimed to be used are actually present in the structures produced. FTIR is based on analyzing the chemical bonds between the molecules of the materials used. In this study, PLGA and PEG polymers were used in the structure of the produced nanoparticles and CUR was selected as a therapeutic agent. As a result of the literature review, specific peaks of CH, CH_2, and CH_3 groups of PEG were observed at 2888 cm^{-1}. Peaks representing the C-H band were observed at 1464 and 1343 cm^{-1}. The O-H and C-O-C bands were observed at 1342^{-1} and 1096 cm^{-1} [25,26]. Singh et al. observed O-H, C-H, C-H, C=O, C-O, and C-H characteristic peaks of PLGA polymer at 3450-3500, 2885-3010, 1762, 1186-1089, and 1450-850 cm^{-1}, respectively [27]. In light of these results, the characteristic peaks belonging to PLGA and PEG used PNP production in this study were observed at 2940, 1461, 1421, 1286, 892 cm^{-1} according to the FTIR results. The specific peaks belonging to CUR were observed at 1630 and 1505 cm^{-1} in the literature, while the peak belonging to C=C and C=O bands were seen at 1630 and 1505 cm^{-1}, and the O-H band was observed at 3200-3500 cm^{-1} [28]. A specific at 1500-1400 cm^{-1} belonging to the C-O elongation of the O-H group was detected. The C-O band of the ether group was obtained

at 1273 cm^{-1}. The bands observed at 713, 808, and 963 cm^{-1} belong to C-H bands [24]. As a result, the presence of CUR in CNPs was proved with peaks at 3365, 1629, 964, and 813 cm^{-1} according to the FTIR results.

According to the SEM results, it was proven that the nanoparticles were produced using PLGA and PEG polymers with the double emulsion solvent evaporation technique. Furthermore, an increase obtained after encapsulating CUR in the particle size were found compatible with the literature [29]. In addition, these nanoparticle sizes were determined as enough small for making them to cross from the blood-brain barrier [30].

4. CONCLUSION

As a result, a new DDS was developed for the treatment of Alzheimer's disease. CUR was preferred as a therapeutical agent for this system due to its various mechanisms in the treatment. By using FTIR analysis, it was proven that PNPs were produced by using PLGA and PEG polymers, and CUR was loaded to these nanoparticles. Furthermore, CNP and PNP morphologies were observed under SEM and the particle sizes of nanoparticles increased after encapsulating CUR to PNP.

5. MATERIALS AND METHODS

5.1. Materials

Polyvinyl alcohol (PVA, Mw ~ 89,000-98,000), polylactic-co-glycolic acid (PLGA, Mw ~ 24,000-38,000), polyethylene glycol (PEG, Mw ~ 4,000), and curcumin were purchased from Sigma-Aldrich (Turkey). All purchased materials were analytical grade.

5.2. Methods

5.2.1. Synthesis of CUR-loaded PLGA/PEG nanoparticles

CUR-loaded PLGA (Acid terminal, lactide:glycolite 50:50, Mw 24000-38000) nanoparticles were prepared by double emulsion solvent evaporation technique (water/oil/water). 40 mg PLGA was dissolved in 2 ml dichloromethane. 50 µg of CUR dissolved in 200 µl PEG was added and homogenized to this solution. Then the mixture was added to 50 ml of PVA (1%, w/v) solution and homogenized to form a water/oil/water emulsion. Excess dichloromethane was removed from the solution using a magnetic stirrer. Nanoparticles were collected by centrifugation at 12000 rpm for 20 minutes. The nanoparticles were then washed three times with deionized water and lyophilized for drying [31].

5.2.2. Fourier transform infrared spectroscopy (FTIR)

FTIR measurements were performed using a Jasco FT/IR 4700 spectrometer and spectrographs were analyzed using OPUS Viewer version 6.5 software to determine the molecular content of the nanoparticles to confirm the presence of PLGA, PEG, and CUR. Measurements were obtained between 500 and 4000 cm^{-1} wave numbers at 4 cm^{-1} resolution at room temperature [32].

5.2.3. Scanning electron microscopy (SEM)

SEM (EVO LS 10, ZEISS instrument) was utilized to investigate the morphology of the nanoparticles. Gold was chosen to cover the surface of all samples for 1 min before SEM. Mean particle size were determined by Zetasizer Nano ZS 90 (Malvern Instruments, Ltd., UK) [32].

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