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ORIGINAL RESEARCH

# Double-Controlled Release of Poorly Water-Soluble Paliperidone Palmitate from Self-Assembled Albumin-Oleic Acid Nanoparticles in PLGA in situ Forming Implant

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**Purpose:** To investigate the effects of solvents on the formation of self-assembled nanonization of albumin-oleic acid conjugates (AOCs) using a solvent exchange mechanism for the construction of in situ forming implants (ISFI).

**Methods:** A poorly water-soluble drug, paliperidone palmitate (PPP), was chosen as the model drug. AOC was synthesized with the 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) reaction. Dichloromethane, tetrahydrofuran, ethanol, N-methyl-2-pyrrolidone, dimethyl sulfoxide, and deionized water were selected to investigate the formation of self-assembled AOC nanoparticles (AONs). The volume ratios of organic solvents against water could determine the miscibility, injectability, and in situ nanonizing capability without aggregation.

**Results:** As the polarity of the organic solvents increased, the AONs exhibited a spherical shape, and the larger the volume of the solvent, the smaller the size of the AONs. To use AOC in ISFI for controlled release of PPP, poly(d,l-lactide-co-glycolide) (PLGA) was combined with the AOC in 2 mL of N-methyl-2-pyrrolidone and water solution (1.8/0.2 ratio). The release rates of all formulations exhibited similar curve patterns overall but were more controlled in decreasing order as follows: AOC, PLGA, and AOC/PLGA for 14 days.

**Conclusion:** A combined formulation of AOC and PLGA was found to effectively control the initial burst release of the drug.

**Keywords:** albumin-oleic acid conjugate, self-assembled nanonization, solvent type, in situ forming implant, solvent exchange, controlled release

## Introduction

To increase the patient's medication compliance in the case of patients with mental disorders or the elderly, who require unmet medical needs for dosage forms, it is very important to study the long-acting injectable (LAI) form of certain medications, such as antipsychotics and macromolecular drugs. Drugs for treating chronic diseases, such as antipsychotics, must maintain an effective concentration in the blood to exhibit long-term efficacy while minimizing unwanted side effects. LAI is a dosage form that lasts for a prolonged period of time with a single administration and is attracting attention because it solves the problem of poor patient compliance and maintains drug concentration for a long time.[1,2] An in situ forming implant (ISFI) is an LAI drug delivery system that utilizes the sol-gel conversion process.

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When an ISFI solution with low viscosity is injected, it readily transforms into rigid and biodegradable implants or depots at the injection site of the body. Moreover, ISFI can be manufactured in a simple process, and the use of large needle gauges or microsurgery could be avoided. Various pharmaceutical strategies exist to trigger ISFI using different transforming principles, such as in situ cross-linking, in situ solidifying organogels, and in situ phase separation.[3] PLGA is an FDA-approved biodegradable and biocompatible polymer for LAI depot formulations. PLGA can be degraded into lactic acid and glycolic acid under physiological conditions, which are biologically inactive within growing cells and can be eliminated from the body through normal metabolic pathways. Therefore, it has been used and studied for a long time in clinical practice due to low systemic toxicity and good biocompatibility.[4,5] Since the first approval of the Lupron Depot using PLGA, in January 1989, by the FDA, over 20 LAI depots have been formulated and marketed for various drugs.[6]

Despite the several advantages of ISFI in the pharmaceutical market, one of the key issues yet to be resolved is the physicochemical variability of the shape and structure of the ISFI and the initial burst release of the drug during implant transformation. The initial burst release is a phenomenon in which the drug is first intensively released within a short period of time after administration. The initial burst release of the drug is usually an undesirable phenomenon because the duration of the drug decreases and toxic effects may occur due to potentially high drug concentrations in the blood.[7,8]

To investigate the feasibility of the new ISFI at the injection site of the body in terms of controlling the initial burst effect and the release rate of poorly water-soluble drugs, fattigation-platform technology was utilized in this study. Fattigation-platform is a technology that forms an amphiphilic structure by constructing an amphiphilic conjugate between a protein drug or a macromolecule and a fatty acid, thereby forming self-assembled nanoparticles, and such technology has been continuously studied in our laboratory.[9,10] It is a very promising technique because biodegradable and biocompatible materials such as albumin, gelatin, and transferrin have been utilized to solubilize poorly water-soluble drugs and aid in the cancer treatment.[9,11–13]

The aim of this study was to investigate the feasibility of employing an amphiphilic albumin-oleic acid conjugate (AOC) for ISFI capability and solubilization of poorly water-soluble drug in a solvent system using solvent exchange mechanisms. Injectable solvents used in ISFI systems must have low viscosity, high water affinity, polymer dissolving ability and non-toxic properties.[14] We examined six solvents with different polarities, namely dichloromethane (DCM), tetrahydrofuran (THF), ethanol, N-methyl-2-pyrrolidone (NMP), dimethyl sulfoxide (DMSO), and deionized water. The self-assembled nanonization of AOC to AOC nanoparticles (AONs) was also studied by characterizing its particle size, zeta potential, using dynamic light scattering (DLS). The surface morphology of AONs was visualized using a field emission scanning electron microscope (FE-SEM), and a field emission transmission electron microscope (FE-TEM). The AOC solution in organic solvent/water was dispersed in different volumes of pH 7.4 phosphate-buffered saline (PBS) solution to observe the self-assembled AON. In addition, PLGA was chosen and combined with AOC to investigate any synergistic effects of ISFI using solvent exchange. Finally, the release rates of various formulations (drug alone, AOC, PLGA, and AOC/PLGA) in NMP/water cosolvent against pH 7.4 PBS solution were measured.

Paliperidone palmitate (PPP), a second-generation antipsychotic, was chosen as the model drug. PPP is the prodrug of paliperidone with a fatty acid conjugate and is used for LAI. Due to its low solubility and hydrophobicity, PPP is released very slowly via degradation of the ester bond by the esterase enzyme present in the muscle, which releases the active paliperidone.[15]

# Materials and Methods

## Materials

Paliperidone palmitate (PPP) was procured from Myeong In Pharm. Co., Ltd. (Seoul, Korea). Human serum albumin, poly (D, L-lactide-co-glycolide) (PLGA), 1–ethyl–3-(3-dimethylaminopropyl) carbodiimide (EDC), and PBS were purchased from Sigma-Aldrich (Saint Louis, MO, USA). Oleate sodium was purchased from Junsei Chemical Co., Ltd (Kyoto, Japan). Methanol, acetonitrile, and alcohol anhydrous of high-performance liquid chromatography (HPLC) grade were purchased from Thermo Fisher Scientific (Waltham, MA, USA). DCM, THF, NMP, and DMSO were purchased from Daejung Chemicals & Metals Co., Ltd. (Seoul, Korea). All other materials were of HPLC grade and used without purification.

## Preparation of AONs

### Synthesis of AOC

AOC was synthesized using the EDC reaction as described earlier.[6] One gram of HSA dissolved in 100 mL of 1% acetic acid and 80 mL of MeOH was used to protonate the free amine of HSA, and then this mixture was stirred at 50 rpm at room temperature (25 °C) for 30 min. Oleate sodium 300 mg and EDC 190 mg dissolved in 100 mL of deionized water were stirred at 500 rpm at 80°C for 30 min to form the O-acylisourea active ester. Then, the protonated HSA solution and activated oleic acid solution were mixed by stirring at 550 rpm at room temperature for 30 min to form the albumin-oleic acid conjugate. The resulting solution was dialyzed for 24 hr using a membrane (3.5 KDa MWCO) against deionized water to remove residue and organic solvent. AOC solution was collected in 50 mL conical tubes. Each sample was frozen at −80°C in a deep freezer overnight and freeze-dried for 48 hr to collect AOC powders.

## Effect of Solvent Type on AOC Nanonization

AOC powder (10 mg) was dispersed in 2 mL of six solvents of different polarities. The polarity indices of various organic solvents are shown in Table 1. The effect of the solvent type on the self-assembled nanonization of AOC and visual images was characterized using DLS and FE-SEM, respectively.

**Table 1** Comparison of the Polarity Index of Organic Solvents

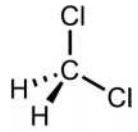
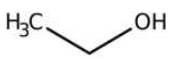
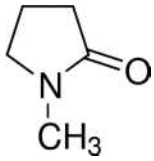
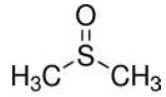
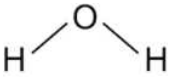
| | Solvent Type | Polarity Index |
|---|---|---|
|  | Dichloromethane (DCM) | 3.1 |
|  | Tetrahydrofuran (THF) | 4 |
|  | Ethanol | 5.2 |
|  | N-Methyl-2-pyrrolidone (NMP) | 6.7 |
|  | Dimethyl sulfoxide (DMSO) | 7.2 |
|  | Water | 10.2 |

**Table 2** AOC Formulations in Different Ratios of Organic Solvents and Water for Solvent Miscibility and Injectability

| Formulation | F1 | F2 | F3 | F4 | F5 | F6 | F7 | F8 | F9 | F10 | F11 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Organic solvent (mL)* | 2.0 | 1.8 | 1.6 | 1.4 | 1.2 | 1.0 | 0.8 | 0.6 | 0.4 | 0.2 | 0 |
| $H_2O$ (mL) | 0.0 | 0.2 | 0.4 | 0.6 | 0.8 | 1.0 | 1.2 | 1.4 | 1.6 | 1.8 | 2.0 |
| AOC (mg) | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 10 |

**Note:** *NMP, DMSO, ethanol, and THF were chosen and used.

**Table 3** Compositions of Drug-Loaded AOC/PLGA Formulations for a Long-Acting PPP Injectable

| | Drug Only | AOC | PLGA | AOC/PLGA | |
|---|---|---|---|---|---|
| | | | | F12 | F13 |
| NMP (mL) | 1.8 | 1.8 | 1.8 | 1.8 | 1.8 |
| Water (mL) | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |
| AOC (mg) | – | 10 | – | 10 | 10 |
| PPP (mg) | 10 | 10 | 10 | 10 | 10 |
| PLGA (mg) | – | – | 100 | 50 | 100 |

## Preparation of AOC Injectable Formulation

### Comparison of AOC Solutions in Different Solvents

To create an AOC solution that forms a depot at the injection site, 10 mg of AOC powder was added to the organic solvents and water in various ratios. Table 2 shows the formulations of AOC (F1–F11) with different ratios of organic solvents (NMP, DMSO, ethanol, and THF) and water for solvent miscibility and injectability. Next, visual images and gelation properties of AOC solution were investigated. Finally, the F2 composition consisting of NMP (1.8 mL), water (0.2 mL), and 10 mg of AOC was chosen and dispersed in 5 mL, 10 mL, or 20 mL of pH 7.4 PBS solution to characterize the physicochemical properties, such as particle size, polydispersity, and zeta potential.

## Preparation of AOC/PLGA Solution

To impart the longer acting role of AOC, PPP-loaded AOC and PLGA solutions in NMP and water were prepared. Table 3 lists the compositions of AOC, PLGA, and AOC/PLGA (F12, F13) formulations in NMP/water solvent. PPP-loaded formulations were then dispersed in 10 mL of pH 7.4 PBS solution for release studies. The visual images and release rates of the ISFI were investigated.

## Characterization of the AOC

### Fourier Transform-Infrared (FT-IR) Spectroscopy

HSA, oleic acid, and AOC powder were investigated using an FT-IR spectrometer (Nicolet iS50, Thermo, Tokyo, Japan). This was done to confirm the conjugation synthesis between albumin and oleic acid. The spectral range set

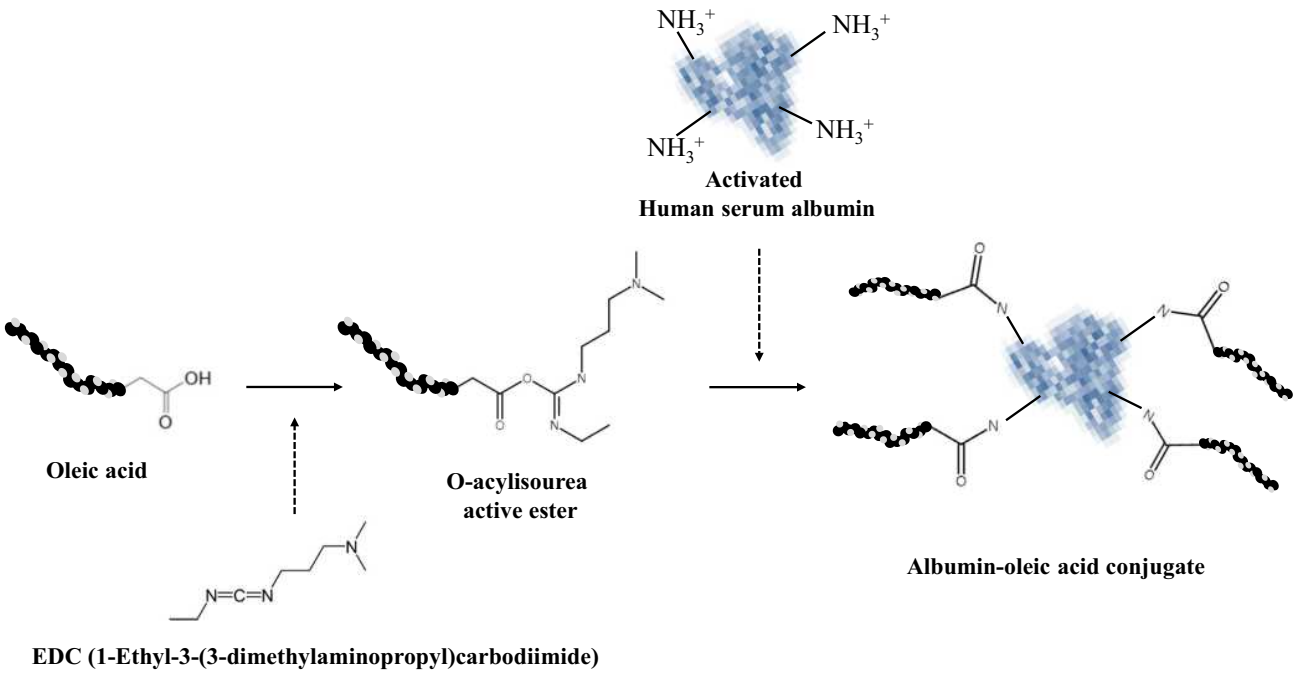



**Figure 1** A schematic representation of the albumin-oleic acid conjugation process using the EDC coupling reaction.

from 400 to 4000 $cm^{-1}$ was recorded at a resolution of 2 $cm^{-1}$.

### Field Emission Scanning Electron Microscope (FE-SEM)

The surface morphologies of the AONs were investigated using FE-SEM (JSM-7900F, JEOL, Tokyo, Japan). It was measured to observe the morphologies of the nanoparticles based on the type of solvent. Twenty microlitre samples were dropped on a copper grid-covered carbon tape. The solution samples were dried for 24 hr and then coated with platinum for 60 s in vacuum.

### Field Emission Transmission Electron Microscope (FE-TEM)

Morphologies of AONs and PLGA combined with AON were investigated using FE-TEM (Tecnai G2 F30 S-Twin, Philips-FEI Corp., Best, Netherlands). Samples were stained with 2% PTA solution for staining. Twenty microlitre samples were then dropped on a TEM copper grid and dried for 24 hr at room temperature.

### Dynamic Light Scattering (DLS)

The particle size and surface zeta potential of nanoparticles were analyzed using ELSZ-2000 S (Otsuka Electronics, Osaka, Japan). All samples were diluted using twice the volume of deionized water and the particle size and surface zeta potential of each sample were analyzed in triplicate.

### In vitro Release Study

In vitro release studies of four different formulations were performed in triplicate using a dialysis kit (Pur-A-Lyzer) and with shaking at 60 rpm. The dialysis kit lid was sealed to prevent evaporation. The dialysis kit was soaked in a conical tube containing 40 mL of pH 7.4 PBS solution. Next, 400 μL of each formulation in NMP/water cosolvent equivalent to 2 mg PPP were dispersed against 2 mL of

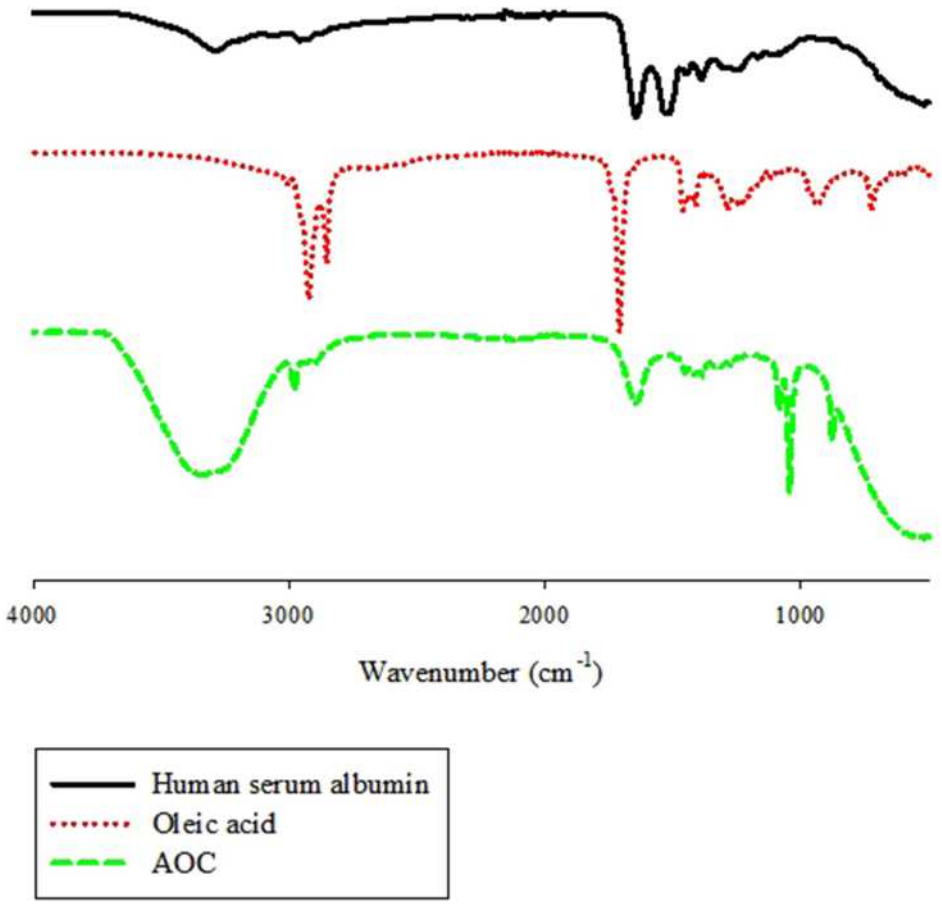



**Figure 2** FT-IR spectra of HSA, oleic acid, and HSA-oleic acid conjugates.

PBS solution. Then, 1 mL of the solution samples were withdrawn for 14 days from the conical tube for PPP analysis using an HPLC system. One millilitre of fresh PBS solution was replenished to maintain sink conditions. A mixture of solution A: solution B (A: B = 25:75, v/v) was used as the mobile phase with a flow rate of 1.0 mL/min. Solution A was composed of 10 mM ammonium acetate in deionized water with 0.1% formic acid, whereas solution B was composed of acetonitrile: isopropanol = 70:30 (v/v) with 0.1% formic acid. Each sample was filtered through a 0.45 μm Rainbow syringe filter (SPT1345B, Hanam, Korea) before injection.

# Results and Discussion

## Synthesis and Characterization of AOC Nanonization

### Identification of AOCs

The mechanism of AOC synthesis is briefly shown in Figure 1. The carboxylic acid group of oleic acid reacted with EDC to form an O-acylisourea active ester, and a new amide bond was formed through an addition reaction with the nucleophilic amine group of HSA. The formation mechanism and identification of AOC have been well recognized in our fattigation researches.[9,11–13] Albumin is an endogenous protein of human blood and is safely used

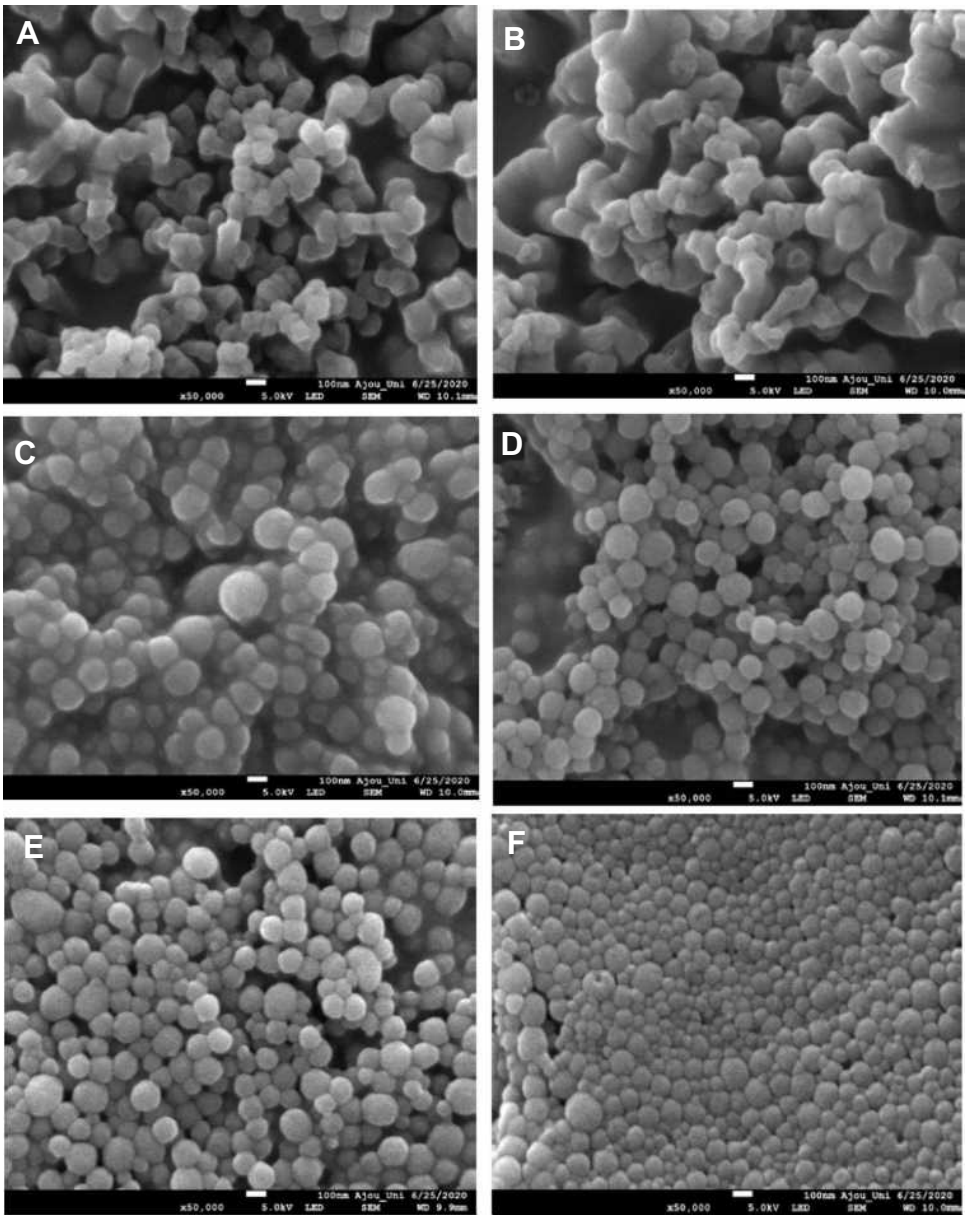



**Figure 3** FE-SEM images of self-assembled AONs in various solvents: (**A**) DCM, (**B**) THF, (**C**) ethanol, (**D**) NMP, (**E**) DMSO, and (**F**) $H_2O$.

as a pharmaceutical excipient in many dosage formulations. Albumin is one of the very attractive substances in nanomedicine, as it has been proven to be successfully applied to Abraxane® in clinical nanomedicine due to the non-toxic and non-immunogenic, ensuring biocompatibility for albumin nanoparticles.[16,17] Oleic acid is also a biocompatible fatty acid.[13] For these reasons, a conjugate of albumin and fatty acid was successfully synthesized, which was also known to be nontoxic and biocompatible.

The HSA-oleic acid conjugates were characterized using FT-IR spectroscopy (Figure 2). The amide bond between albumin and oleic acid was confirmed. The broad-spectrum band was confirmed from 3100 to 3500 $cm^{-1}$ in the spectra of AOC due to N-H stretching vibration, indicating that an amide bond occurred between albumin and oleic acid. Because of its biodegradability and biocompatibility, albumin has the potential for a wide range of biomedical applications.[18] Moreover, oleic acid is an unsaturated fatty acid, which is a biocompatible substance widely distributed in plasma.[19]

### The Effect of Organic Solvents on AOC Nanonization

To investigate the effect of various solvents on the formation of self-assembled AONs, they were formed by dispersing AOC powder in six solvents of different polarities. In various solvents, the shape and morphology of AON varied, comprising aggregated, rectangular, or spherical formats. As the polarity of the solvent increased, the shape of the nanoparticles became more uniform (Figure 3). It is presumed that as the polarity of the solvent increases, the hydrophobic oleic acid portion of AOC assembles to form a spherical shape. Table 4 shows the effect of solvent type on the physicochemical properties of self-assembled AONs according to the polarity index of the solvents, which are particle size (nm), polydispersity index, and zeta potential (mV). DLS showed that particle size exhibited various values regardless of the polarity of the solvent. It is known that physical properties of nanoparticles, including shape and size, vary depending on the surrounding environment, especially on the solvent environment.[20]

## Solvent Exchange and Self-Assembled Nanonization of AOC Formulations

### Preparation and Visual Images of AOC Solution

To investigate the feasibility of the in situ forming depot of the AOC solution at the injection site, AOC powder was dispersed in a solution of organic solvents (not DCM) and water at different ratios (F1–F11). Figure 4 shows dispersion images of AOC at various ratios of (a) NMP, (b) DMSO, (c) ethanol, and (d) THF solvent, with water, visualizing clear solution, turbid solution, and AOC gelled particles, depending on the types of cosolvents tested. To examine the detailed physical state of the solution for the solvent ratios of 2 mL of NMP and water (F3, F4, and F5), FE-SEM images of the AOC gel formation for three different ratios of 2 mL of NMP/water are shown in Figure 5. There were floating gelled particles in these solutions. This AOC gel was rigid and could not be injected, nor could it pass through the needle, although AOC powder was soluble in the NMP solvent. It is well known that NMP is a common solvent and drug vehicle that enhances the solubility of poorly water-soluble drugs. However, drug-loaded AOCs dispersed in water or NMP/water were nanonized to form AONs without aggregation.

AOC solution in 2 mL of NMP/water (F2: 1.8/0.2) was dispersed in different volumes of pH 7.4 PBS solution to observe the self-assembled AONs, assuming that pH 7.4 PBS formation is the injection site, which is a hydrophilic environment. The visual images of the AOC formulation in NMP/water cosolvent (F2: 1.8/0.2) after dispersing in different volumes of pH 7.4 PBS solution are shown in Figure 6. The solutions appeared clear and slightly turbid. Table S1 shows the stability of AONs after dispersion of the AOC formulation (F2) in 2 mL of NMP/water with

**Table 4** Effect of Solvent Type on the Physicochemical Properties of Self-Assembled AONs

| | Polarity Index | Particle Size (nm) | Polydispersity Index (P. I.) | Zeta Potential (mV) |
|---|---|---|---|---|
| DCM | 3.1 | 508.75 ± 19.73 | 0.133 ± 0.248 | −30.14 ± 3.07 |
| THF | 4 | 279.63 ± 38.88 | 0.154 ± 0.011 | −24.45 ± 1.45 |
| Ethanol | 5.2 | 226.4 ± 2.12 | 0.281 ± 0.028 | −55.86 ± 2.66 |
| NMP | 6.7 | 258.61 ± 3.96 | 0.128 ± 0.005 | −44.17 ± 0.51 |
| DMSO | 7.2 | 223.75 ± 5.44 | 0.141 ± 0.037 | −11.63 ± 1.89 |
| $H_2O$ | 10.2 | 194.3 ± 11.03 | 0.164 ± 0.053 | −15.04 ± 3.50 |

**Note:** Mean ± standard deviation (n = 3).

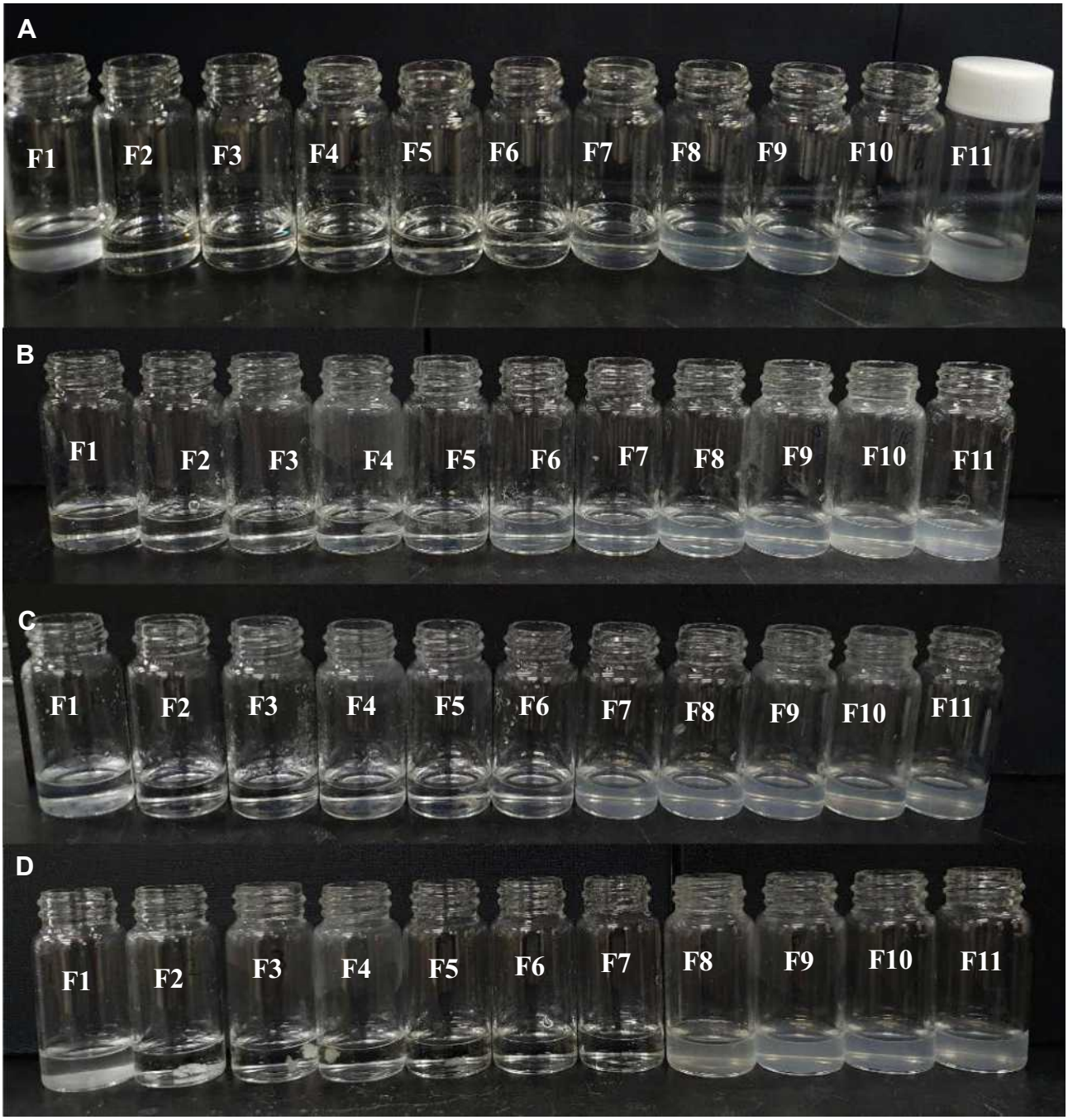



**Figure 4** Dispersion images of AOC in various ratios of water with (**A**) NMP, (**B**) DMSO, (**C**) ethanol, and (**D**) THF solvent.

different volumes of PBS solution, and its stability at room temperature after 2 weeks. The stability of AON was dependent on the volume of the pH 7.4 PBS solution. In general, as the volume of the PBS solution increased, the particle size of the NPs decreased. However, the particle size of the AONs was more stable in 10 mL of PBS solution. In addition, the stability of AOC formulations in terms of polydispersity and zeta potential showed that the AOC formulation (F2) in 2 mL of NMP/water was stable in 10 mL of PBS buffer (Table S1). This result indicated that the physical properties of AONs are affected not only by the type of solvent but also by the volume of solvent. However, an ISFI or depot with AOC alone was unable to form ultimately.

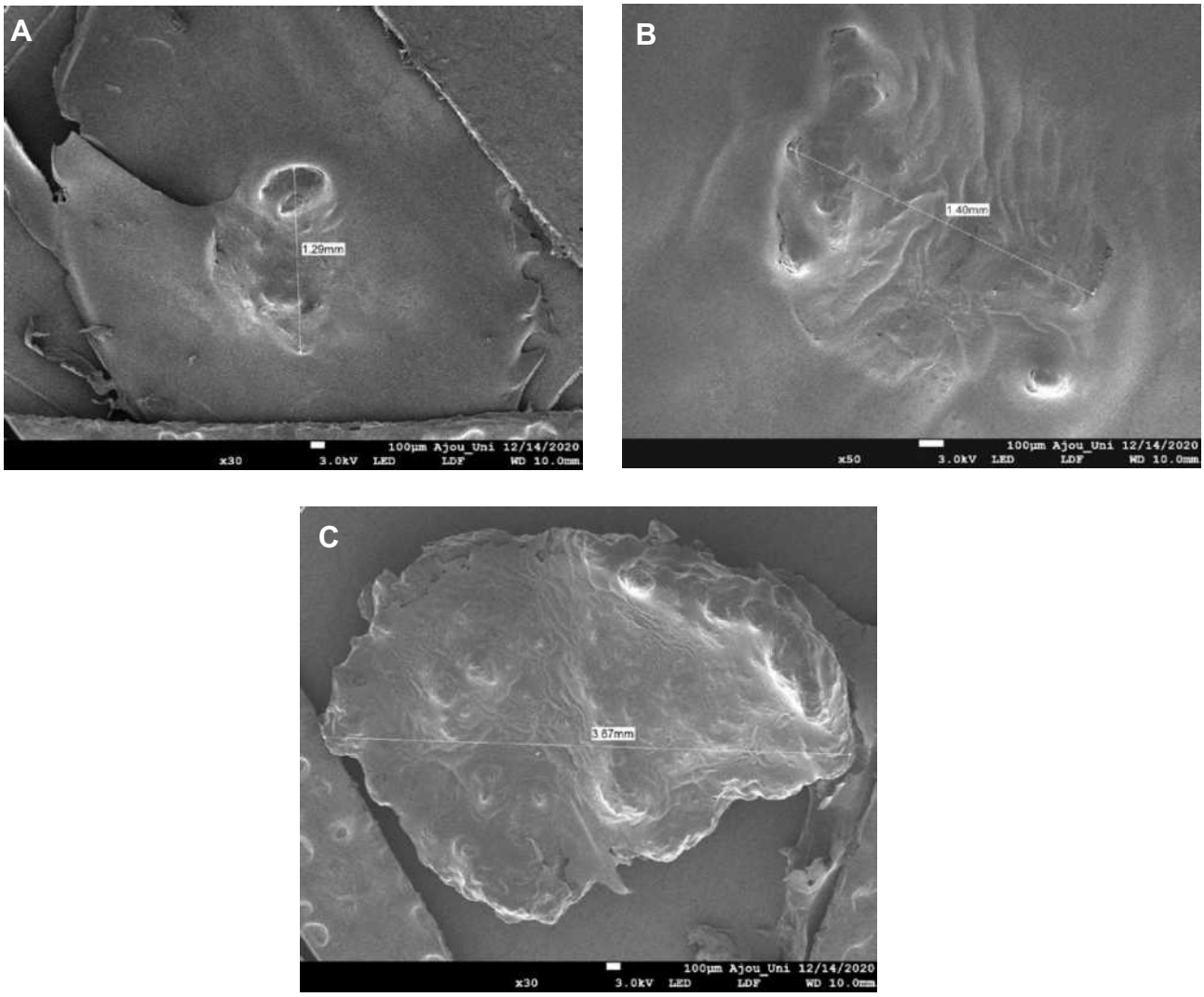



**Figure 5** FE-SEM images of AOC gel formed at three different ratios of NMP/water cosolvent: (**A**) F3: 1.6/0.4, (**B**) F4: 1.4/0.6, and (**C**) F5: 1.2/0.8.

## Preparation of a Long-Acting AOC/PLGA Solution

To prepare a double-controlled long-acting depot formulation, PLGA was combined with AOC solution to enable ISFI using solvent exchange mechanism when administered into the body. Considering that the toxicity and biocompatibility of solvents and polymers is an important factor, the materials used in this study could be said to be appropriate for the development of an in situ implant formulation. PLGA is an FDA-approved biodegradable polymer that enables controlled drug release.[21] Since PLGA has good solubility in NMP solvent, AOC formulations (F2) in 2 mL of NMP (1.8 mL) and water (0.2 mL) were optimally selected due to their good injectability and miscibility without aggregation and gelation before injection, allowing solubilization of AOC and PLGA simultaneously. Furthermore, AONs that were formed using the AOC solution (F2) in NMP/water were deemed stable NMP (N-methyl-2-pyrrolidone) is a polar aprotic solvent frequently used in the pharmaceutical industry. NMP is classified as Class II and FDA-approved solvents at a dose of 5.3 mg/day. For this reason, ISFI having 1.8 mL of the NMP solvent used per injection was a formulation that can be managed sufficiently according to regulatory guidelines. Within established dosages, NMP solvents are effective in dissolving PLGA and maintaining dispersion of nanoparticles, making them a common solvent for in situ forming implant formulations for long-lasting drug delivery.[22,23]

To investigate the effect of PLGA content combined with AOC, we dispersed AOC/PLGA formulations, F12 (50 mg PLGA), and F13 (100 mg PLGA) in 10 mL of pH 7.4 PBS solution. Figure 7 shows the effect of PLGA


**Figure 6** Visual images of AOC formulation in NMP/water cosolvent (F2: 1.8/0.2) after dispersing in different volumes of pH 7.4 PBS solution.

content in AOC/PLGA formulations on the drug depot after dispersion in different volumes of pH 7.4 PBS solution. Unfortunately, F12 AOC/PLGA formed a suspension rather than a depot, but F13 was able to form a depot. For this reason, the F13 AOC/PLGA formulation in 2 mL NMP/water was selected as an optimal ISFI formulation as a "double-controlled" LAI.

To clarify the physical nature of the AOC/PLGA depot formulation, morphological images after dispersing in PBS solution were observed using FE-TEM during storage both initially and 2 weeks later (Figure 8). When measured immediately after dispersion, a spherical shape was shown with AONs loaded on the PLGA network (Figure 8A). After 2 weeks, AONs were gradually released as PLGA was disrupted and eroded (Figure 8B). These morphological changes were also validated by checking the physical state of the depot-forming AOC/PLGA formulation. Table 5 shows the physical stability of the AOC/PLGA formulation (F13) in 2 mL of NMP/water cosolvent at room temperature for 2 weeks after dispersion in 10 mL of PBS solution. The particle size increased by more than two-times at 2 weeks post-dispersion compared to initially because of the gradual degradation of the PLGA network, which liberated AON nanoparticles. In contrast,

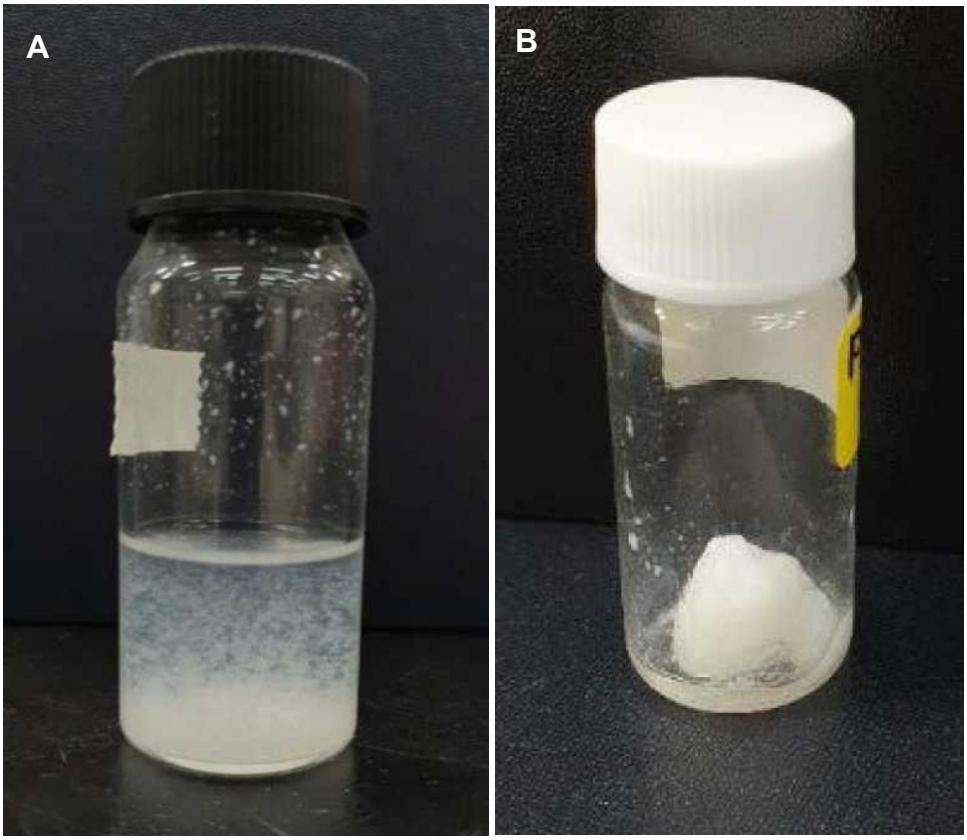



**Figure 7** Effect of including PLGA in AOC/PLGA formulations on drug depot formation after dispersion in different volumes of pH 7.4 PBS solution. (**A**) F12 (50 mg), (**B**) F13 (100 mg).

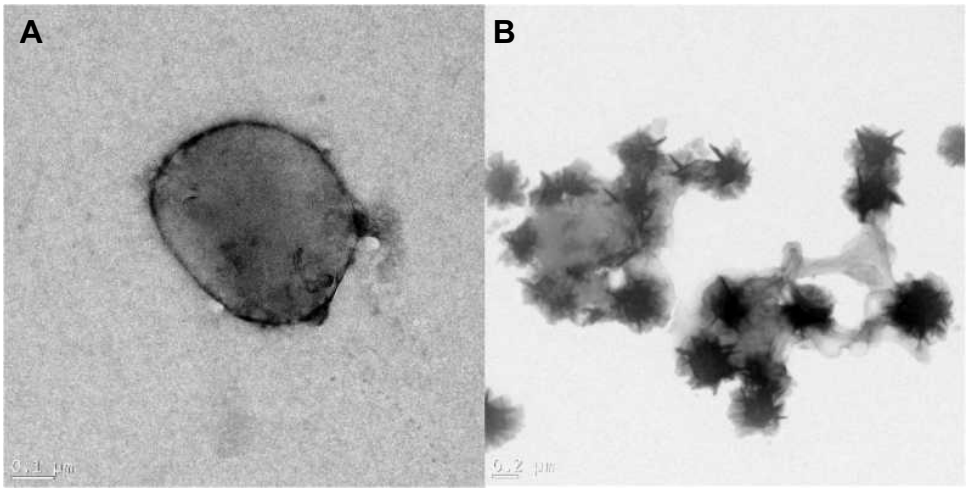



**Figure 8** FE-TEM images of AOC/PLGA formulation (F13) during storage. (**A**) Initial and (**B**) after 2 weeks.

other parameters such as the polydispersity index and zeta potential were almost unchanged. Based on these results, it was demonstrated that PLGA was gradually destroyed and AONs were released.

## In vitro Release Profile of AOC and AOC/PLGA Formulation

The in vitro release profiles of AOC or depot-forming AOC/PLGA formulations using solvent exchange mechanisms were studied. Figure 9 shows the controlled release profiles of PPP from the various formulations in NMP/water cosolvent in pH 7.4 PBS solution at 37°C and with shaking at 60 rpm. The release rates of all formulations were overall similar but were more controlled in decreasing order as follows: AOC, PLGA, and AOC/PLGA, compared to PPP alone for 14 days because of the low solubility of PPP. PPP alone showed an initial burst effect in which more than 50% of the drug was released in the first 24 hr. The PPP-loaded AOC formulation also showed controlled release from AONs via AOC nanonization-encapsulating PPP with less than 40% of the drug released during the first 24 h, followed by gradual release

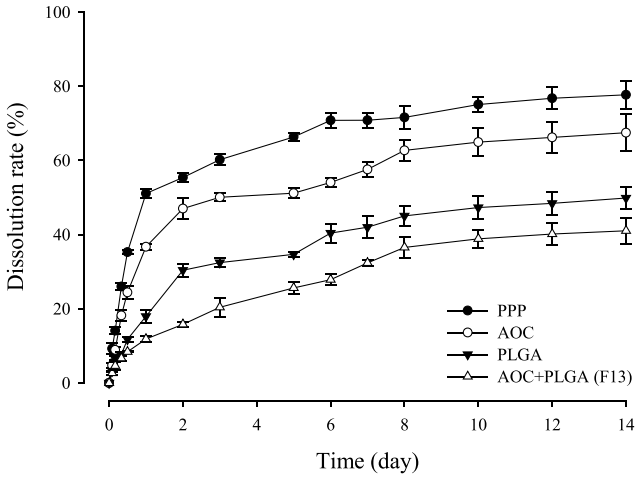



**Figure 9** Controlled release profiles of PPP from the various formulations in NMP/water cosolvent against pH 7.4 PBS solution.

thereafter. PPP-loaded PLGA formulations also exhibited a more controlled release pattern than the AOC alone. Interestingly, the combined formulation using AOC/PLGA showed more synergistic and double-control effects of the drug release with the aid of the nanonizing AOC and sustaining PLGA; furthermore, only 11% of the drug was released on the first day, minimizing the initial burst effect. Beyond the first day, it exhibited drug release over 14 days in a well-controlled manner.

Based on these findings, the release mechanism of the ISFI formulation that comprises nanonizing AOC and depot-forming PLGA is described in Figure 10. AOC was very unique to control the solubility and release rate by dispersing poorly water-soluble drug in the amphiphilic network of the self-assembled nanoparticles. This optimal

**Table 5** Physical Stability of the AOC/PLGA Formulation (F13) at Room Temperature in 2 mL of NMP/Water Cosolvent at 2 Weeks Post-Dispersion in 10 mL of PBS Solution

| | Initial | 2 Weeks |
|---|---|---|
| Particle size (nm) | 617.6 ± 17.4 | 1.695.6 ± 158.3 |
| Polydispersity Index (P.I.) | 0.264 ± 0.002 | 0.357 ± 0.11 |
| Zeta potential (mV) | −9.38 ± 0.04 | −14.47 ± 0.17 |

**Note:** Mean ± standard deviation (n = 3).

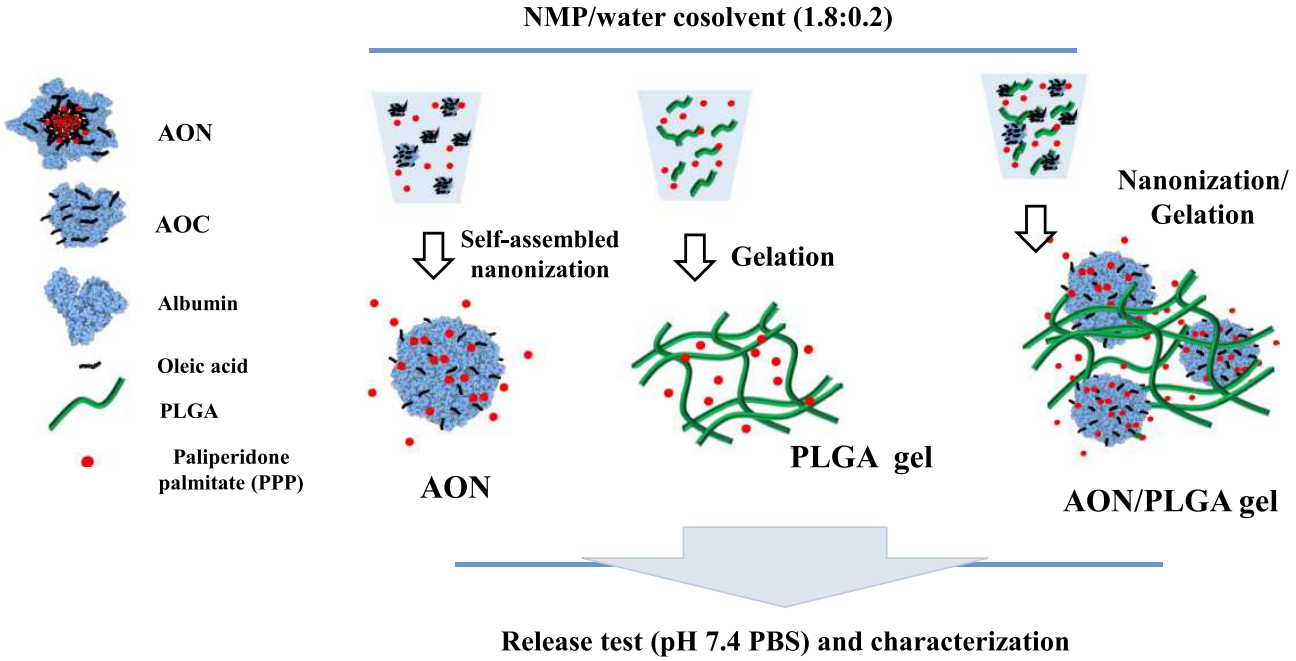



**Figure 10** The release mechanism of ISFI formulation comprising nanonizing AOC and depot-forming PLGA.

ISFI formulation could lead to a "double-controlled" LAI of PPP using a PLGA polymeric network and nanonizing AOC. Furthermore, the combined formulation of AOC and PLGA in the development of LAI was found to effectively control the release rate without showing the initial burst release of the poorly water-soluble drug.

## Conclusions

AOC was successfully synthesized via the conjugation of albumin and oleic acid. Various solvent types could influence the formation of AONs via self-assembled nanonization of AOC. The effect of six solvents of different polarities on the formation of self-assembled AONs was observed by studying the different morphological changes. To investigate the feasibility of the ISFI of the AOC, the NMP/water cosolvent was tested after dispersion in PBS solution but was ultimately unable to form a depot. Instead, we found that a combination of a nanonizing AOC and depot-forming PLGA in 2 mL of the NMP/water cosolvent system could provide a double-controlled PPP via solvent exchange and self-assembled nanonization of AOC in PLGA polymeric network for ISFI. This ISFI could effectively modulate the release rate of PPP in a controlled manner for 2 weeks, minimizing the initial burst effect of PPP. The AOC fattigation-platform combined with PLGA polymers in specific solvent systems could serve as a useful technology for the ISFI design of LAIs.

## Abbreviations

PPP, Paliperidone palmitate; LAI, Long-acting injectable; ISFI, In situ forming implants; AOCs, albumin-oleic acid conjugates; AONs, AOC nanoparticles; EDC, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide; PLGA, Poly-d,l-lactide-co-glycolide; PBS, Phosphate-buffered saline; FT-IR, Fourier transform infrared spectroscopy; DLS, Dynamic light scattering; FE-SEM, Field emission scanning electron microscopy; FE-TEM, Field emission transmission electron microscope; DCM, Dichloromethane; THC, Tetrahydrofuran; NMP, N-methyl-2-pyrrolidone; DMSO, Dimethyl sulfoxide.

## Data Sharing Statement

The data presented in this study are available upon request.

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## Disclosure

The authors declare that there are no conflicts of interest regarding the publication of this study.

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