A Novel Poly(DL-lactic acid) Nanoparticle of Nitroxide Derivative, 4-Ferrocenecarboxyl-2,2,6,6-tetramethyl Piperidine-1-oxyl

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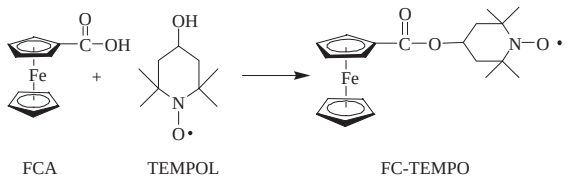
A newly synthesized nitroxide radical, 4-ferrocenecarboxyl-2,2,6,6-tetramethyl piperidine-1-oxyl (FC-TEMPO), was firstly encapsulated in the poly(DL-lactic acid) (PLA) nanoparticles by the modified spontaneous emulsification solvent diffusion method. The formation of the nanoparticles with diameters of 120 ± 15 nm has been confirmed by a laser particle size analyzer. The morphology of the nanoparticles exhibited a fine spherical shape without aggregation or adhesion by the scanning electron microscopy (SEM). The electron paramagnetic resonance (EPR) spectra indicated that FC-TEMPO was mainly encapsulated into PLA nanoparticle core.

Low molecular weight piperidine nitroxides are widely used as a spin label in the biological and pathological areas. Piperidine nitroxides can be used in noninvasive methods to monitor the drug release and pO_2 measurement in tissues through electron paramagnetic resonance (EPR) and magnetic resonance imaging (MRI).[1] At the same time, studies indicate that some piperidine nitroxides as antioxidants can exert cytoprotective action against diverse oxidative stress induced by radiation, cytotoxic drugs, and so on. Furthermore, piperidine nitroxides could suppress tumorigenesis as the cell proliferation modifier and apoptosis inducer.[2] However, the nitroxides are reduced rapidly to hydroxylamines in tissue specimens.[3] For example, nitroxides are reduced to the corresponding diamagnetic hydroxylamines inside the body by reducing agents such as ascorbic acid and thioredoxin reductase. In order to avoid a direct contact of the nitroxides with the surrounding tissue and consequently lose the paramagnetism, several approaches such as the inclusion of nitroxides in implants or the microencapsulation of the nitroxides in liposomes or microspheres have been described.[4] These systems can protect the nitroxide from bioreduction to hydroxylamines, deliver the drug to the desired site of action in the body and decrease or avoid the side effect at non-target site.[5] Poly(DL-lactic acid) (PLA) has been widely developed as microsphere/nanoparticle carrier for drug-delivery systems due to its desirable biocompatible and biodegradable properties. To our best knowledge, there is no report that the piperidine nitroxide was encapsulated in PLA nanoparticles as mode drugs. In this paper, a newly synthesized piperidine nitroxide derivative, 4-ferrocenecarboxyl-2,2,6,6-tetramethyl piperidine-1-oxyl (FC-TEMPO), was entrapped in PLA nanoparticles with a narrow size distribution. FC-TEMPO could exert cytotoxic effects by induction of apoptosis on cancer cells, which was developed by our patent.[6] The PLA nanoparticle carrier of FC-TEMPO may provide new functions for further drug-release investigantions of piperidine nitroxides and their noninvasive technology applications.

The FC-TEMPO was synthesized by the ester bond of ferrocenecarboxylic acid (FCA) and 4-hydroxy-2,2,6,6-tetramethyl-piperidine-1-oxyl (TEMPOL) according to the patent.[6] FC-TEMPO was orange crystal (mp 175–177 °C). MS (ESI): 384.1260. IR(KBr): 3103.25, 2919.78, 2850.90, 1462.52, 1373.95, 1282.90, 1139.93 cm^{-1}. Elemental analyses: Anal. Calcd for $C_{20}H_{26}FeO_3N$: C, 62.56; H, 6.82; N, 3.65%. Found: C, 62.62; H, 6.90; N, 3.39%. EPR (toluene): $g = 2.0063$. The structure of three compounds was shown in Scheme 1.

FC-TEMPO loaded PLA nanoparticles were prepared according to the modified spontaneous emulsification solvent diffusion (SESD) method.[7] The typical procedure was as follows: An amount of PLA (80 mg), span (100 mg), and FC-TEMPO (3 mg) were codissolved in 20 mL of mixed organic solvent consisting of acetone/ethanol (1:1, v/v) with the aid of ultrasound. The mixture solution was then added dropwise into 40 mL of the aqueous solution containing 0.5% (w/v) of sodium dodecyl sulfate (SDS) using a peristaltic pump at a flow rate of 0.5 mL/min while continuously stirring at 600 rpm with a propeller mixer. Organic solvents were evaporated from the dispersion system under reduced pressure. The final volume of emulsion was adjusted to 30 mL and aggregates were removed by means of the filtration. The produced nanoparticles were collected by ultracentrifuge (16000 rpm; 25 min; 4 °C) and washed with distilled water at least three times to remove the surfactant. The obtained suspension was used for analysis or freeze-dried to obtain the white powdered nanoparticles. The nanoparticle product was stored at 4 °C.

In the modified-SESD process, the mixture of two water-miscible organic solvents (methanol/acetone) was employed to form the PLA nanoparticle. The mechanism of the nanoparticle formation is probably similar to the one speculated by the reference.[8] When the PLA solution consisting of binary solvents is dispersed into the aqueous SDS solution, the perturbation of the interface spontaneously produces a larger interfacial area, which leads to nano-sized quasi-emulsion droplets of PLA solution. First, methanol preferentially diffuses from the droplets due to its lower affinity to PLA than acetone. And then, acetone diffuses from the droplets and the coacervation of SDS is induced. In addition, the PLA condenses, deposits, and solidifies. These



FCA TEMPOL FC-TEMPO

Scheme 1.

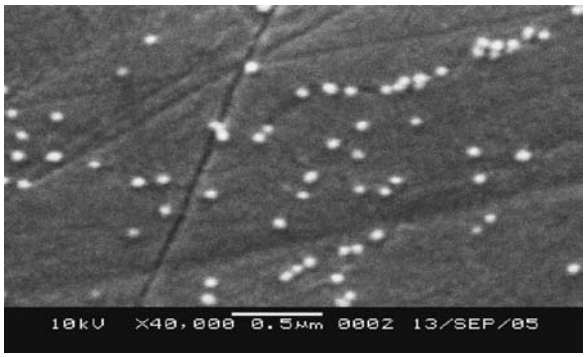


Figure 1. The SEM of FC-TEMPO-loaded PLA nanoparticles prepared with the methanol/acetone (1:1) system.

instantaneous and spontaneous deposition processes can lead to the uniform nanoparticle dispersion even by mild agitation, and finally form the PLA nano-sized patrticles.

FC-TEMPO-loaded nano-sized particles of PLA can be effectively obtained by dropping the polymeric organic solution into an aqueous phase with moderate mechanical stirring. The mean diameter of the PLA nanoparticles in emulsion was 120 ± 15 nm by a laser particle size analyzer (ZERAS12ER-2000, Malvent). The size distrubition of particles was narrow and the nanoparticles were reproductive.

The scanning electron microscopy (SEM) was used to characterize the morphology of the FC-TEMPO–PLA nanoparticles and to give evidence of encapsulation. SEM measurements were performed on a XL30 (Philip) electron microscope. The results showed that the morphology of the nanoparticles exhibited a fine spherical shape without aggregation or adhesion in Figure 1.

The determination of drug loading was assayed using a UNICO UV-2102PC spectrophotometer. The powdered nanoparticles were dissolved in dichloromethane and the solution was determined spectrophotometrically at 454 nm for FC-TEMPO. The encapsulation efficiency (EE) of FC-TEMPO in the powdered nanoparticles was calculated as the reference.[7] The 89% ee indicated that the higher content of drug was incorporated in PLA carriers. Additionally, the proportion of the drug incorporated into the nanoparticle core was also studied by the EPR method.

As shown in the inset of Figure 2, EPR spectrum of pure FC-TEMPO solid exhibited the single-line signal. However, the EPR peak of FC-TEMPO nanoparticle powder stored for 2 months at 4 °C, which loaded the same amount of drugs as pure solid, was too small to be measured at the same condition. After measurement, the result shows that the recovery yield of FC-TEMPO nanoparticles stored for 2 months at 4 °C was approximately 85%. When the FC-TEMPO nanoparticles stored for 2 months were dissolved in dichloromethane, the EPR spectrum of FC-TEMPO nanoparticles was three-line EPR signal that was similar to the spectrum of the solution of pure FC-TEMPO as shown in Figure 2. The close similarity between the EPR spectra of the two systems suggests that the PLA coating had no effects on the properties of FC-TEMPO in the nanoparticle core, and FC-TEMPO stably existed in the PLA nanoparticle cores at least for 2 months at 4 °C. Moreover, the primary result shows that the PLA coating can protect FC-TEMPO against

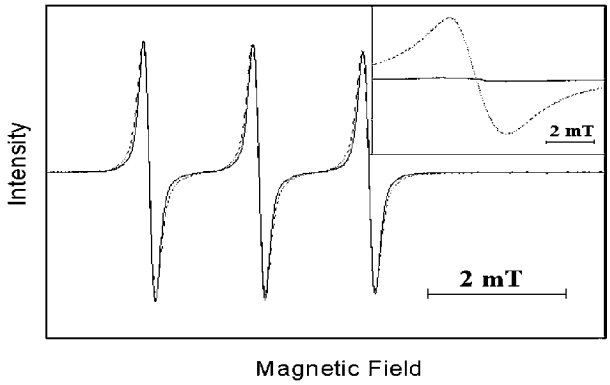


Figure 2. The Comparison of EPR spectra of pure FC-TEMPO (- - -) and the FC-TEMPO-loaded nanoparticles stored for 2 months (—) in dichloromethane. Inset shows EPR spectra of pure FC-TEMPO solid (- - -) and FC-TEMPO-loaded powder nanoparticles stored for 2 months (—) under atmospheres. Curves are normalized by the amount of FC-TEMPO with the encapsulation efficiency calculation.

reduction by ascorbic acid.

In conclusion, a newly nitroxide derivative FC-TEMPO was firstly encapsulated in the PLA nanoparticles by the modified SESD method with high encapsulation efficiency. The FC-TEMPO-loaded PLA nanoparticles were discrete spherical shape and the drugs were mainly entrapped in the PLA nanoparticle core by the EPR assay. The obtained PLA nanoparticle carrier of FC-TEMPO may provide a base for further drug-release investigantions of piperidine nitroxides and their noninvasive technology applications.

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