

CONTROLLED DRUG RELEASE ANALYSIS OF MONOSIZED DRUG-LOADED PLGA MICROPARTICLES BY LIGAND-SENSITIZED FLUORESCENCE

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ABSTRACT

A drug release monitoring method using Tb³⁺ ion as a fluorescent probe of controlled drug release system with monosized drug-loaded poly(lactic-co-glycolic acid) (PLGA) microparticles is presented. The luminescence properties of Tb³⁺-enoxacin complex were characterized by 3D fluorescence (FL) spectrum to optimize the instrumental parameters. Drug release profiles from the enoxacin-loaded monodisperse PLGA microparticles were simultaneously monitored by the presented FL method and the UV-Vis spectroscopy for the evaluation of the signal correlation. By using this novel FL method, the effects of solvents on the drug release profile of drug-loaded monodisperse PLGA microparticles were investigated.

KEYWORDS

PLGA microparticle, ligand-sensitized fluorescence, controlled drug release, microfluidic flow focusing device.

INTRODUCTION

Recently, manipulating techniques for the controlled (or sustained) drug release have gained tremendous interests in drug delivery system (DDS) and micro total analysis system (μ -TAS) for pharmaceutical or biotechnological applications. [1-2] Considerable research has been conducted to investigate DDS via biodegradable and biocompatible polymeric carriers. Among the polymer materials, PLGA has been widely employed as a matrix for encapsulation of biomolecules and synthetic drugs. Conventionally, the drug release profile is determined by measuring the absorbance of drug molecules released into release medium by UV-Vis spectrophotometry [3] and high performance liquid chromatography (HPLC) [4]. Other techniques involve measuring the FL intensity of the fluorescent molecule itself [5], not the drug. In this study, we prepared enoxacin (ENX)-loaded monodisperse PLGA microparticles using a microfluidic flow focusing device (MFFD) followed by a solvent evaporation technique. The prepared ENX-loaded PLGA microparticles were then used to investigate the drug release profiles via a novel detection method, Tb³⁺ as a fluorescent probe. This method was designed to monitor drug concentration in release medium more sensitively and to make the measurement procedure simple.

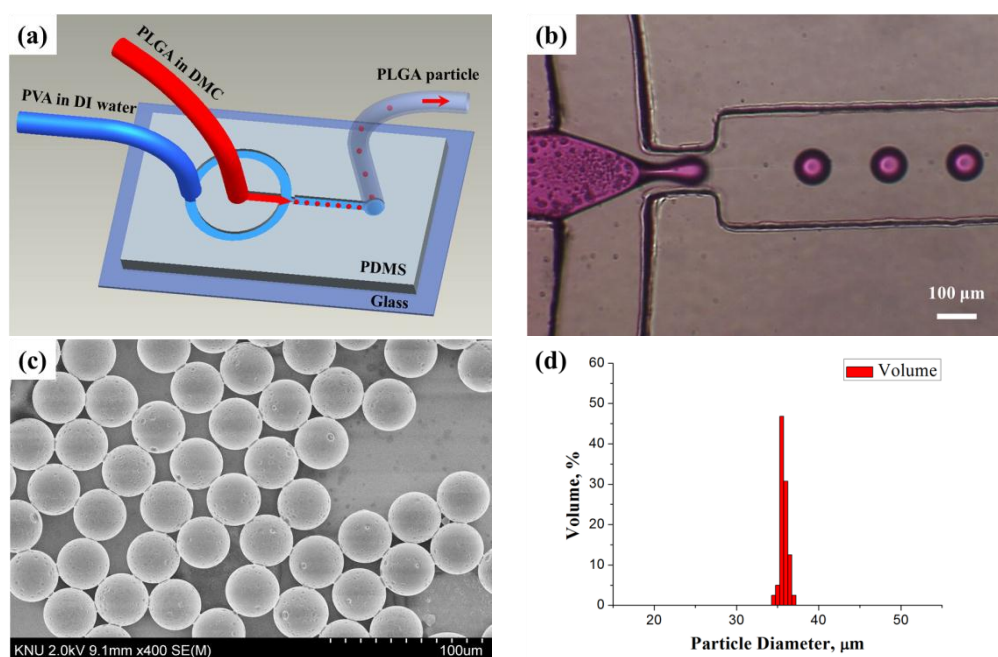


Figure 1. The monodisperse PLGA microparticle fabrication using a microfluidic flow-focusing device and its characterization (a) schematic diagram of a MFFD, (b) inverted optical microscopy image of the flow-focusing region generating microdroplets, (c) SEM image of the PLGA microparticles and (d) size distribution.

EXPERIMENT

A drug, ENX that is fluoroquinolone antibiotics, loaded PLGA microparticles were prepared by a microfluidic chip-based flow focusing device (Figure 1(a, b)). Different from bulk and membrane emulsification methods, the microfluidic chip-based flow focusing device produces nearly monosized microparticles as shown in Figure 1(c, d). Furthermore, the diameter of the monodisperse PLGA microparticles could be adjustable from 30 to 50 μm by controlling the flow rate of disperse and continuous phase. Because it is important for quantitative research to precisely control the size distribution and diameter which are deeply related with PLGA degradation process. The mean diameter of the PLGA microparticles was 36.7 μm with the standard deviation of 0.95 μm ($n=178$). Several micro-craters induced by solvent diffusion between the oil-water interfaces were observed on the surface of the PLGA microparticles.

When Tb^{3+} ion makes complex with organic compound such as β -diketone or polycarboxylic acid, the FL intensity is highly enhanced. The enhancement of FL intensity can be explained by intramolecular energy transfer from ligands to metal ion, which is widely known as ligand-sensitized FL or antenna effect (Figure 2(a)) [6-7]. Figure 2(b) shows characteristic sharp FL emission peaks of Tb^{3+} complex due to its 4f orbital transition. Two different calibration curves of ENX were acquired by measuring absorbance at wavelength of 268 nm and emission FL at 544 nm using ENX standard solution in order to convert each signal to ENX concentration (Figure 2(c, d)). The presented FL method was analyzed and compared with UV-Vis absorption method as shown in Table 1. The results showed that the presented FL method produced more sensitivity with lower detection limit than the UV-Vis method. Under the optimized parameters, drug release kinetic profiles from ENX-loaded PLGA microparticles were simultaneously cross checked by presented FL and absorbance in order to investigate signal correlation between two methods (Figure 3).

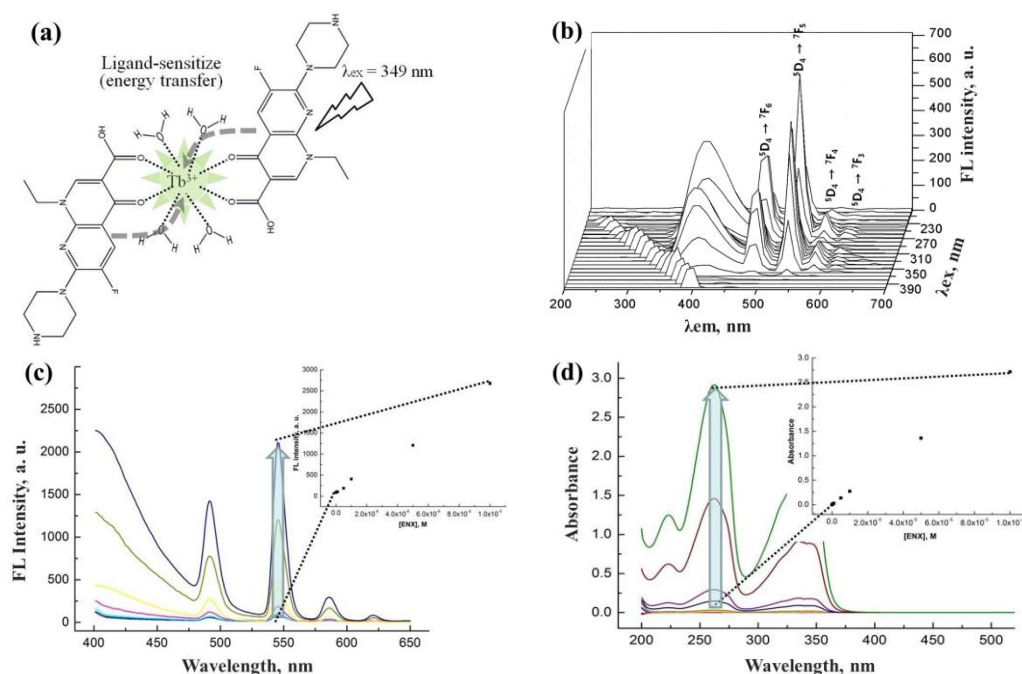


Figure 2. Drug release detection method (a) conceptual schematic illustration of energy transfer from ENX to Tb^{3+} , (b) 3D fluorescence spectrum of Tb^{3+} -ENX complexes, calibration curves of ENX using (c) ligand-sensitized FL and (d) UV-Vis method.

Table 1. Comparison between ligand-sensitized FL and UV-Vis method

Data analysis	Ligand-sensitized FL	Abs
Calibration curve (x, μM)	$y = 253.5x + 79.6$	$y = 0.272x + 0.001$
Dynamic range (μM)	0.01~10	0.05~100
Detection limit (nM)	1.5	9.8
Reproducibility (%)	2.80	1.59
Correlation coefficient	0.9974	0.9994

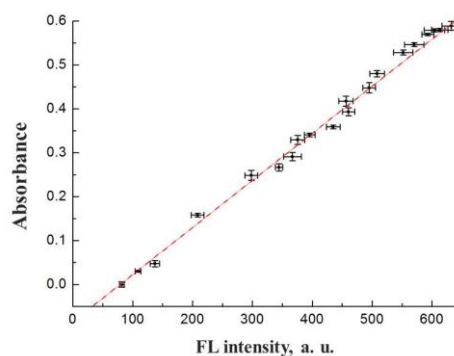


Figure 3. Signal correlation between ligand-sensitized fluorescence and absorbance in drug release system.

To investigate a solvent effect on drug release, two solvents of dichloromethane (DCM) and dimethyl carbonate (DMC) were adopted to dissolve PLGA in the preparation procedure of ENX loaded PLGA microparticles. Then, the drug release kinetic profiles of two kinds of ENX loaded PLGA microparticles were monitored over 47 days as shown in Figure 4. Although the burst release induced by diffusion was observed with both conditions, those synthesized with DMC showed that slowly increased concentration range from 10 to 32 day by degradation of PLGA. The degradation process of ENX-loaded PLGA microparticles (Figure 4(b, c)) showed physical morphology with porous microstructure that influence drug releasing rate.

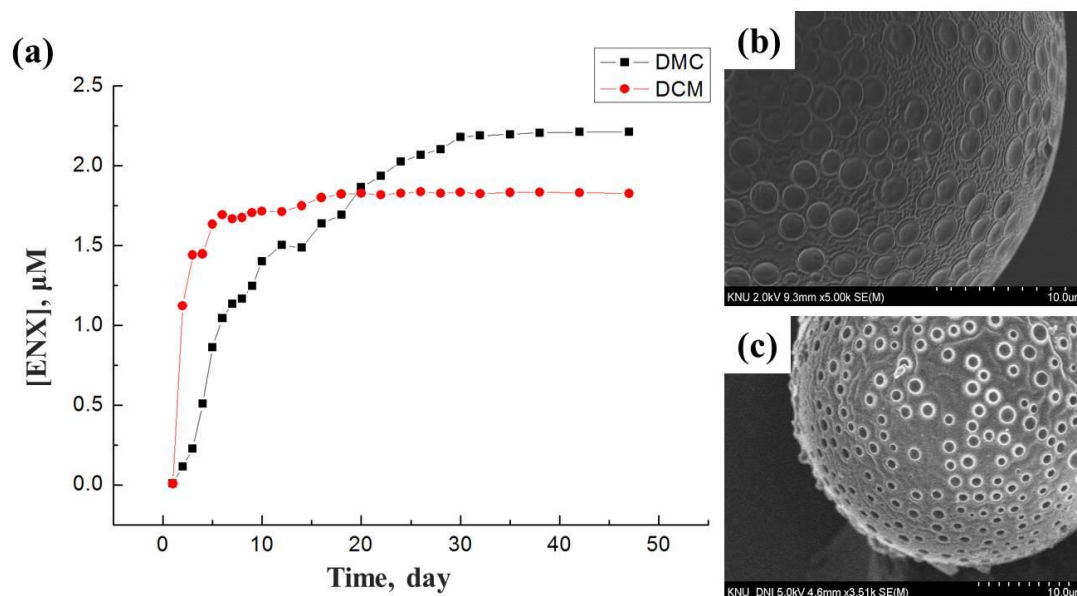


Figure 4. (a) Cumulative ENX release profile from PLGA microparticles, surface morphology of degradable PLGA microparticles (b) day 1, (c) day 47.

CONCLUSION

The drug encapsulated PLGA microparticles were prepared by MFFD to keep diameter and size distribution of microparticles under control. Moreover the ligand-sensitized FL, Tb^{3+} as fluorescent probe, was successfully applied to drug release monitoring from the prepared PLGA microparticles. The presented FL method provide superior way for drug release monitoring because of its lower detection limit as well as better sensitivity than UV-Vis method, furthermore the presented FL method can be introduced to various other drugs and biomolecules which make complex with lanthanide ions.

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