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Effect of a surface stabilizer on the formation of polyoxalate nanoparticles and their release profiles

Nutthakritta Phromviyo [a], Ekaphan Swatsitang [a,b], Apiwat Chompoosor [a,b,*]

[a] Materials Science and Nanotechnology Program, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
[b] Department of Physics, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand

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ABSTRACT

Biodegradable polyoxalate nanoparticles coated with polymeric surface stabilizer were successfully fabricated using an emulsion method. A series of biocompatible polymers such as Poly(vinyl alcohol) (PVA), poly(vinylpyrrolidone) (PVP) and polyethylene glycol (PEG) were used as surface stabilizers. The effects of surface stabilizers on the morphologies of nanoparticles were investigated. Polymeric stabilizers at various concentrations were used to study the formation of polyoxalate nanoparticles (POX-NPs). Scanning electron microscopy (SEM), transmission electron microscopy (TEM), dynamic light scattering (DLS), and zeta potential were employed to characterize the resulting POX-NPs. The results demonstrated that surface stabilizers greatly influenced the particle sizes and surface properties of POX-NPs. PVP and PVA potentially can be used as surface stabilizers in the formulation of biodegradable polyoxalate. The size, surface and release properties of POX-NPs can be effectively controlled by varying the preparation conditions.

1. Introduction

Biodegradable polymeric nanoparticles have been used extensively as drug delivery systems due to their superior properties that include high bioavailability, high drug loading and minimal toxicity. These nanocarriers can be preferentially accumulated in tumors through the enhanced permeation and retention (EPR) mechanism. When the nanocarriers have been localized in the tumor, they can release drugs over a period of time and minimize the side effects of these drugs [1–5]. Moreover, they can be degraded into smaller molecules and excreted from the body with minimal toxicity. Various biodegradable polymers have been used to formulate nanoparticles [6–8]. Biodegradable polymers including poly(D,L-lactide), poly(lactic acid), poly(D,L-glycolide), poly(lactide-co-glycolide), and poly(cyanoacrylate) have been used to prepare nanoparticles in recent years [4]. Polyoxalate is a biodegradable polymer and is considered a potential drug carrier. Polyoxalate has been suggested for several medical uses such as absorbable sutures. It can be easily synthesized by a condensation reaction between diols and oxalyl chloride [9]. Polyoxalates can be hydrolytically cleaved into non toxic smaller molecules. Additionally, they are highly sensitive and chemical labile to hydrogen peroxide at nanomolar concentrations [10–12]. Therefore, polyoxalates are degradable in the intracellular environment. Due to their biodegradability and biocompatible characteristics, polyoxalate have attracted interest as biodegradable nanoparticles to carry therapeutic agents.

Several methods can be used to prepare these nanoparticles such as spray drying, extrusion and supercritical fluid extraction [13,14]. Emulsification solvent evaporation has been widely used for synthesis of nanoparticles. In this technique, polymeric nanoparticles are formed by evaporating the organic solvent in which the polymer is dissolved. A stabilizer is usually added to the formulation to protect the emulsion formed during the particle preparation process [15]. It also can prevent particle aggregation and facilitate formation of smaller sized particles. Smaller particle size results in greater surface area as well as improved solubility and absorption of nanoparticles [16]. Moreover, nanoparticles have enhanced cellular uptake compared to microparticles [17–20].

Polyoxalate has a promising potential for use in drug delivery applications. However, little work has been done to investigate the use of polyoxalate to formulate nanoparticles and their ability to release therapeutic agents in a controlled manner. The current

* Corresponding author. Materials Science and Nanotechnology Program, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand.
E-mail address: Apiwat@kku.ac.th (A. Chompoosor).

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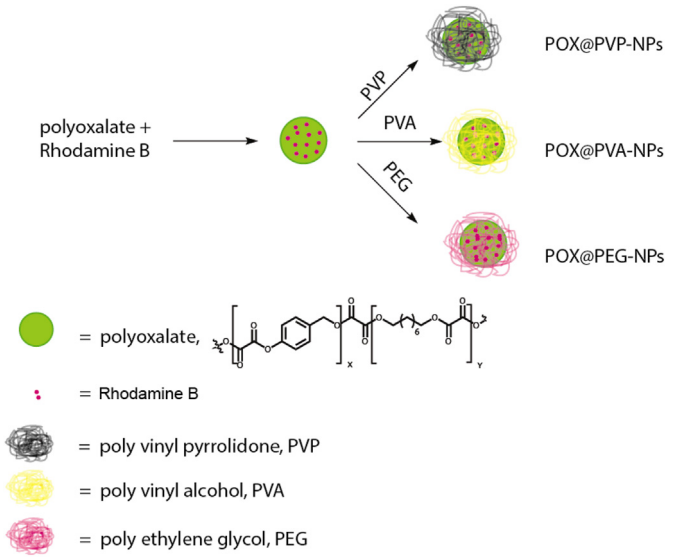

Fig. 1. Schematic illustration of the formation of POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs by the emulsification solvent evaporation method.

study is aimed to investigate the effects of surface stabilizers on the preparation of polyoxalate nanoparticles (POX-NPs) and their release profile *in vitro*. The results showed that surface stabilizers greatly influenced the particle size and release profile of POX-NPs. PVP and PVA have potential uses as surface stabilizers to formulate biodegradable polyoxalate. This work demonstrates the potential use of POX-NPs in drug delivery vehicles.

2. Materials and methods

2.1. Materials

Poly(vinyl alcohol) (PVA) (99% hydrolyzed; typical M_w = 27,000), poly(vinyl-pyrrolidone) (PVP) (M_w = 40,000), poly(ethylene glycol) (PEG) (M_w = 8000), and rhodamine B were purchased from Sigma–Aldrich (MO, U.S.A.). Phosphate buffer saline (PBS) buffer was also obtained from Sigma–Aldrich and used without further purification. Polyoxalate (prepared from 1,8-octanediol, 4-Hydroxybenzyl alcohol and oxalyl chloride) was synthesized in our laboratory. Its molecular weight (M_w) and polydispersity (PD) as determined by gel permeation chromatography were 16,000 and 2.72, respectively.

Biodegradable polyoxalate nanoparticles were fabricated using an emulsification solvent evaporation method. Poly(vinylpyrrolidone) (PVP), poly(vinyl alcohol) (PVA), and poly(ethylene glycol) (PEG) were selected as polymer stabilizers to yield POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs, respectively (Fig. 1). These polymer stabilizers are widely used for the synthesis of polymeric nanoparticles because of their biodegradability and biocompatibility [4,21–24]. Rhodamine B, a drug model, was encapsulated and used to monitor the release characteristics of POX-NPs.

2.2. Synthesis of polyoxalate

Polyoxalate was synthesized by a condensation polymerization of 1,8-octanediol and oxalyl chloride. 1,8-octanediol (21.96 mmol) and 4-hydroxybenzyl alcohol (5.49 mmol) were dissolved in 20 ml of dry tetrahydrofuran (THF) under a nitrogen atmosphere and then, oxalyl chloride (27.45 mmol) in 25 ml of THF was added dropwise at 4 °C. The reaction was kept at room temperature overnight. Polymers were extracted by dichloromethane (DCM) and washed with sodium bicarbonate and sodium chloride solutions. Hydrophilic molecules were isolated and filtered by anhydrous sodium sulfate through a glass funnel. Polymer was concentrated under vacuum. The molecular weight was determined by gel permeation chromatography (GPC) using polystyrene standards.

2.3. Preparation of a polymer solution and POX-nanoparticles

Polyoxalate and rhodamine B were dissolved in dichloromethane at concentrations of 5 wt.% and 1 wt.%, respectively at

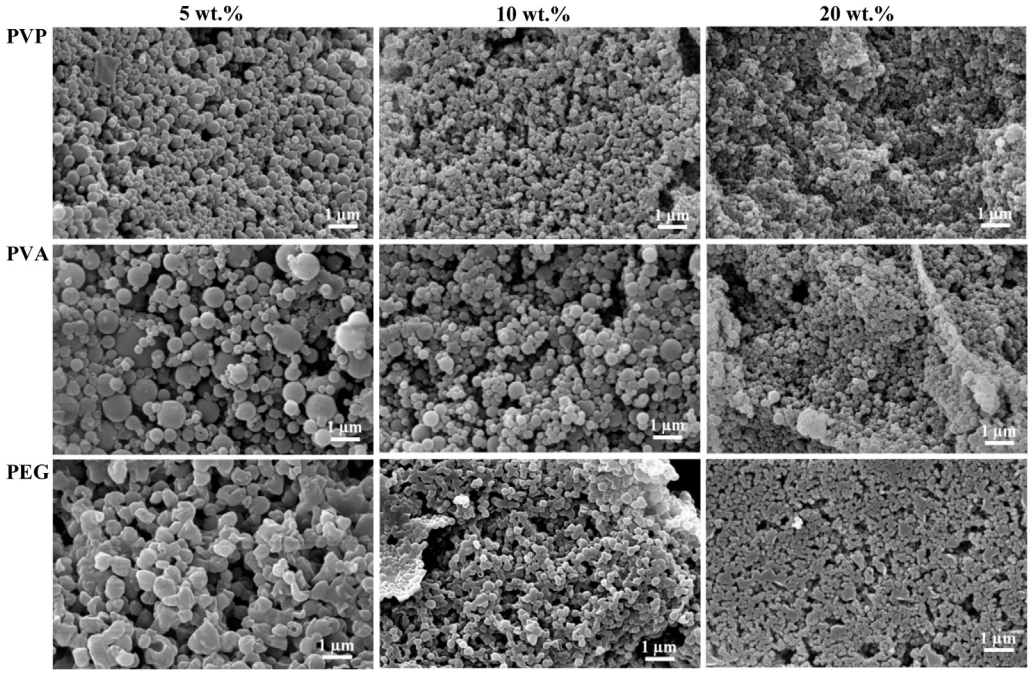

Fig. 2. SEM micrographs of POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs at concentrations of 5 wt.%, 10 wt.% and 20 wt.%.

Table 1
Particle sizes and agglomeration status of POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs at concentrations of 5 wt.%, 10 wt.% and 20 wt.%.

Nanoparticles		Average diameter (nm)	Agglomeration status
POX@PVP-NPs	5 wt.%	280 ± 74	No agglomeration
	10 wt.%	160 ± 57	No agglomeration
	20 wt.%	120 ± 31	No agglomeration
POX@PVA-NPs	5 wt.%	660 ± 83	No agglomeration
	10 wt.%	280 ± 71	No agglomeration
	20 wt.%	200 ± 48	No agglomeration
POX@PEG-NPs	5 wt.%	650 ± 81	Agglomeration nanoparticles
	10 wt.%	200 ± 64	Agglomeration nanoparticles
	20 wt.%	140 ± 61	Agglomeration nanoparticles

25 °C. The solution was then added to an aqueous solution of surface stabilizer, PVP, PVA and PEG at concentrations of 5, 10 and 20 wt.% at 4 °C. Then, the mixture was sonicated (Fisher Scientific, Sonic Dismembrator 500) and homogenized (PRO Scientific, PRO 200) to form an oil/water emulsion. The emulsion solution was poured into water and homogenized again to form a fine oil/water emulsion. The solvent was removed by evaporation. Nanoparticle pellets were obtained after centrifugation, twice washing with de-ionized water, and freeze drying the remaining solid material.

2.4. Characterizations

The morphology and the surface structure of POX-NPs were observed using scanning electron microscopy (SEM) (LEO VP1450, UK) and transmission electron microscopy (TEM) (FEI 5022/22 Tecnai G2 20 S-Twin, CR). Average hydrodynamic size and polydispersity of the nanoparticles were determined using a dynamic light scattering system (DLS) (Malvern Instruments, UK) at 25 °C. The suspension of nanoparticles was measured at concentration of 0.1 mg/ml. For the *in vitro* release test, rhodamine B released from nanoparticles was measured using a spectrofluorometer (JASCO spectrometer FP-8200 equipped with a Xe lamp, Japan). Nanoparticles containing rhodamine B (5 mg) were incubated at 37 °C in 5 ml of phosphate buffer (pH-7.4). After the desired incubation time, the amount of rhodamine B released into the buffer solution was determined and 5 ml of fresh buffer solution was added to the sample and further incubated. Excitation wavelength was set at 500 nm. The emission wavelength was recorded at 582 nm. Fluorescent intensity of rhodamine B was converted to its concentration using a calibration curve of rhodamine B in the phosphate buffer. Then, the cumulative mass and relative percentage of rhodamine B released were determined as a function of incubation time.

3. Results and discussion

PVP, PVA and PEG in concentrations of 5–20 wt.% were used to investigate the effects of stabilizer concentration on the formation of POX-NPs. SEM images of POX-NPs prepared using PVP, PVA and PEG at 5, 10 and 20 wt.% are shown in Fig. 2 and Table 1. As seen in this figure, POX@PVP-NPs are spherically shaped with smooth surfaces at all stabilizer concentrations. Mean particle sizes were 280 nm, 160 nm and 120 nm for concentrations of 5, 10 and 20 wt.%, respectively. POX@PVP-NPs show a narrow particle size distribution. Their mean particle size was found to decrease with increasing PVP concentration. In the case of POX@PVA-NPs made with PVA concentrations of 5, 10 and 20 wt.%, the resulting particles were also round shaped. Their particle sizes were approximately 660, 280 and 200 nm, respectively. Particle size decreased with increasing PVA concentration. POX@PVA-NPs also had a narrow particle size distribution. At high PVA concentration, POX@PVA-NPs showed more discrete nanoparticles. For POX@PEG-NPs, the average particle size also decreased with increasing PEG concentration. The particle sizes were approximately 650, 200 and 140 nm for 5, 10 and 20 wt.% PEG, respectively. However, the POX@PEG-NPs were found to agglomerate when PEG concentration was increased. Agglomeration of POX@PEG-NPs is clearly seen in Fig. 3. These TEM images show POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs at a concentration of 10 wt.%. This result shows that all types of stabilizers had an effect on the morphologies of POX-NPs. POX@PVP-NPs and POX@PVA-NPs are still spherically shaped, while agglomeration of POX@PEG-NPs can be observed.

Fig. 4 shows the comparison of polyoxalate nanoparticle size and PDI using DLS measurements. At 5 wt.% of concentration, the results show that POX@PVA-NPs and POX@PEG-NPs had larger particle sizes of about 660 and 650 nm, respectively. When stabilizer concentrations were increased, the particle sizes resulting from these conditions decreased greatly, from 660 to 650 to 280 and 200 nm, respectively. The particle sizes of POX@PVP-NPs gradually decreased.

As seen from these results, the concentration of stabilizer has an influence on particle sizes of POX-NPs. High concentration of stabilizer yields smaller sized POX-NPs. Particle size is directly proportional to emulsion droplet size and coalescence during hardening. The emulsion droplet size is primarily determined by the degree of shear and amount surface stabilizer used. An increase in the amount of stabilizers used reduced the droplet size and in turn reduced the final particle size. This is consistent with the study of Quintanar-Guerrero et al. [25]. They studied the effect of stabilizers concentration on poly(lactic acid) nanoparticles from propylene carbonate.

Agglomeration of POX@PEG-NPs may be due an inability of PEG to be flexible enough to shield nanoparticles [26]. Furthermore, PEG

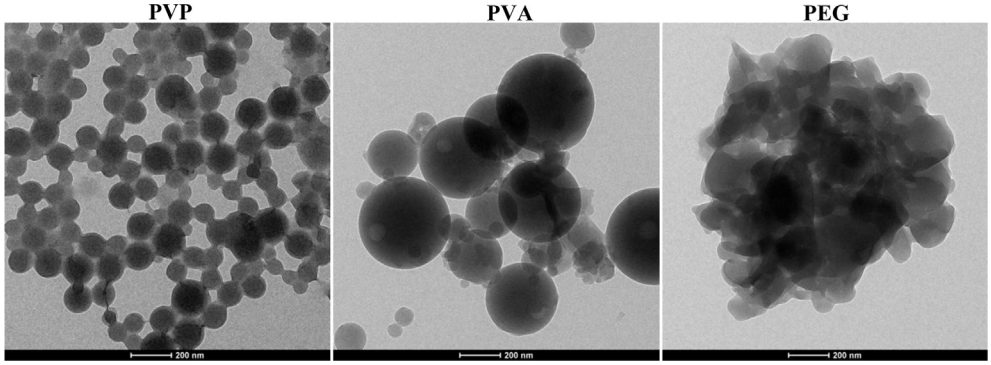

Fig. 3. TEM images of POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs at a concentration of 10 wt.%.

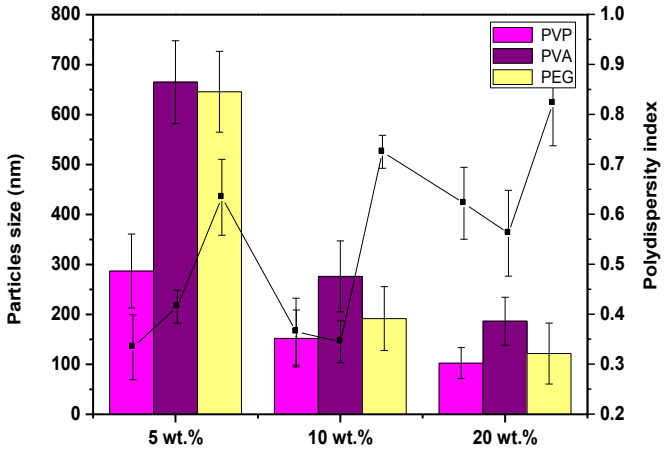

Fig. 4. Particle sizes and PDI of POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs at concentrations of 5 wt.%, 10 wt.% and 20 wt.%.

is quite soluble in water. Thus, it is widely used to improve the solubility of nanoparticles in water [27]. PEG has low amphiphilic behavior due to an imbalance between its hydrophilic and hydrophobic parts. PVP and PVA do not have this imbalance. Accordingly, adsorption of PEG on the nanoparticle's hydrophobic surface is low. POX-NPs prepared using PEG as a stabilizer generate low steric repulsion interactions between nanoparticles. Therefore, POX-NPs prepared with PEG tend to agglomerate. In POX@PVP-NPs and POX@PVA-NPs, PVA and PVP molecules adsorb onto the surfaces of the nanoparticles. The polymeric chain is adsorbed onto the hydrophobic polyoxalate and stretches its hydrophilic moiety into the water phase. Hence, they can generate good steric repulsion interaction between particles. They show high potential stabilization to nanoparticles against aggregation of POX-NPs.

To evaluate release profiles of these nanoparticles, rhodamine B was encapsulated in POX-NPs. Then *in vitro* solubility tests were carried out in phosphate buffer saline (pH ~7.4) at 37 °C to mimic physiological conditions. Fig. 5 shows rhodamine B release from POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs at different stabilizer concentrations. From Fig. 5a–c, it is seen that cumulative rhodamine B release profiles showed increased release with increasing stabilizer concentration. Fig. 5d shows the rhodamine B release of POX-NPs prepared using different types of surface stabilizer at 20 wt.%. It was found that the cumulative release rate of rhodamine B from POX@PVP-NPs was the fastest. The cumulative rhodamine B release of POX@PVA-NPs was the slowest. This occurred for two reasons. First, as shown in the SEM images of Fig. 2, the particle size of POX-NPs decreased when the concentration of stabilizer was increased, giving rise to greater surface area [2,28]. Therefore, the surface area of the POX-NPs increased as the concentration of the stabilizer increased. As a result, the encapsulated rhodamine B in smaller sized POX-NPs exhibited a higher cumulative release than the larger sized POX-NPs. Second, as the concentration of surface stabilizer increased, more surface stabilizer was absorbed onto the POX-NPs. Due to the hydrophilic property of surface stabilizers, POX-NPs with higher concentrations of surface stabilizer adsorbed on their surfaces could effectively degrade and release rhodamine B at a faster rate.

4. Conclusions

In conclusion, we successfully synthesized biodegradable polyoxalate nanoparticles using an emulsion evaporation method. The biocompatible polymers, Poly(vinyl alcohol) (PVA), poly(-vinylpyrrolidone) (PVP) and polyethylene glycol (PEG), were selected as surface stabilizers to form nanoparticles. The types and concentrations of stabilizers were studied. It was found that as surface stabilizers, PVP, PVA, and PEG have a remarkable influence on the particle size and surface properties of POX-NPs. It was demonstrated that PVP and PVA can potential be used as surface stabilizers to effectively synthesize biodegradable polyoxalate. By

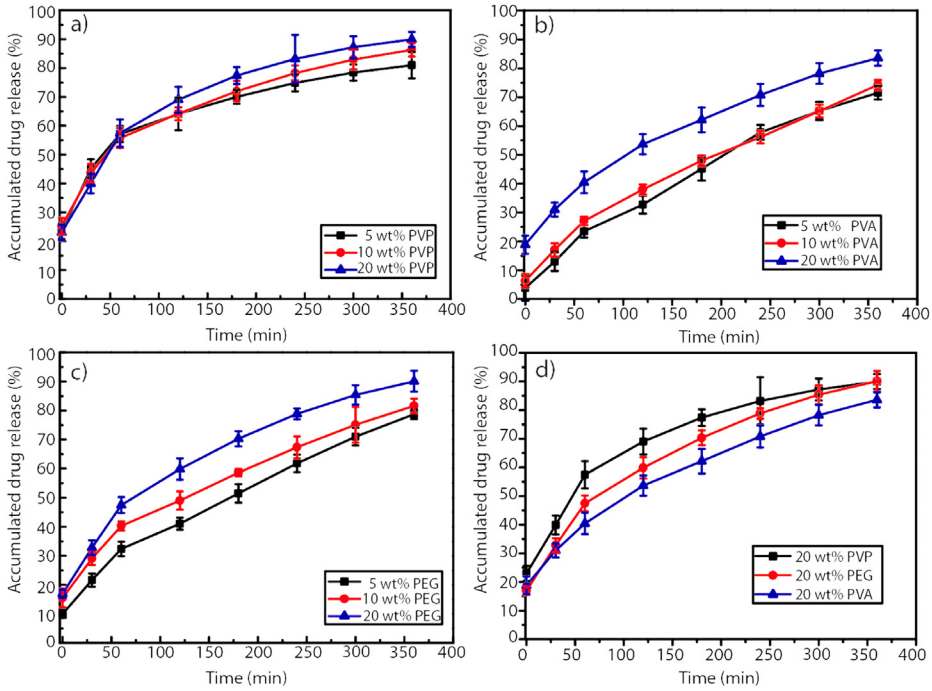

Fig. 5. Rhodamine B release from of a) POX@PVP-NPs at 5 wt.%, 10 wt.% and 20 wt.% b) POX@PVA-NPs at 5 wt.%, 10 wt.% and 20 wt.% and c) POX@PEG-NPs at 5 wt.%, 10 wt.% and 20 wt.% and d) POX@PVP-NPs, POX@PVA-NPs and POX@PEG-NPs at 20 wt.% only.

tuning the preparation conditions, particle size and surface properties of POX-NPs can be effectively controlled.

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