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Honokiol loaded PLGA nanoparticles with modified surface by chitosan

KEYWORDS: Polymer nanoparticles, PLGA, chitosan, honokiol

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ABSTRACT *Polymer nanoparticles have become a powerful tool to deliver and to improve effect of anticancer drugs. Recent widespread studies have used poly lactic-co-glycolic acid (PLGA) as useful material to entrap water-insoluble anticancer drugs. However, using PLGA remains some disadvantages such as its low zeta potential, poor mucoadhesivity and permeability which lead to unstable nanoparticle system, low efficiency of drug absorption through gastrointestinal (GI) tract. In this study, PLGA nanoparticles loading honokiol, an insoluble anticancer drug, were improved its quality by modified surface with chitosan (CS), and CS is a mucoadhesive compound contains positive charge, to form PLGA-CS nanoparticles (PLGA-CS NPs). These nanoparticles were prepared by emulsion solvent diffusion method followed by optimized process. Size distribution, zeta potential, morphology and encapsulation efficiency (EE) are measured to evaluate quality of PLGA-CS NPs. Optimized nanoparticles reached zeta potential of +43 mV as well as 253 nm in average of size. PLGA-CS NPs were sphere indicated by SEM images with high homogeneity and polydispersity index (PdI) was 0.070. EE was about 65%. These stable characteristics proclaimed that PLGA-CS nanoparticles were promising insoluble-drug delivery system to conduct further experiments in vivo.*

1. Introduction

In recent years, nanoparticles have become one of the most promising delivery systems to significantly enhance efficacy of antitumor drugs [1]. Among them, polymeric nanoparticles have received increasing attention due to their ability in improving circulation time and efficiency of drug, reducing drug efflux from cells mediated by multi-drug resistance mechanisms and an amendable surface to chemical conjugation for targeting purposes [2, 3].

One of the most successfully developed polymers used for forming nanoparticles is poly lactic-co-glycolic acid by its attractive characteristics such as biodegradability and biocompatibility [4-6]. However, PLGA NPs needed to improve for applications in oral administration due to its'poor mucoadhesive ability and unstable zeta potential [7]. Ideal drug delivery system for oral administration not only has to reach high zeta potential in order to become stable system in solution and low pH environment of gastrointestinal (GI) tract [1, 8] but also has acceptable mucoadhesive capacity, high permeability as well as smaller than 500 nanometer in diameter to ensure drug delivering through GI barriers [9, 16]. One of methods to increase zeta potential and mucoadhesivity of PLGA NPs is its surface modified by chitosan.

Chitosan is a natural biodegradable polysaccaride derived from chitin. With approximation of 6.5 pKa on the amine groups, chitosan is water-insoluble and gains positive charge in acidic environment [10, 11]. Artursson et al. 1994 and Dodane et al. 1999 reported that positive charge of chitosan reduces the trans-epithelial electrical resistance of cell monolayers [27,28] and chitosan simultaneously increases para-cellular permeability [29]. This cationic polysaccharide has been attractive for researchers to apply in pharmacy and biomedicine because of its abundant capacities such as biocompatibility, biodegradability, nontoxicity and low-immunogenicity [12–14], especially in oral administration. Chitosans' mucoadhesivity is significantly remarkable characteristic. By creating physical barriers to diffusion and interacting electrostatically with cationic molecules, presence of mucus affects free drugs' permeability and uptake of particulates [1], thus mucoadhesivity of chitosan in the form of nanoparticles facilitates free drug to penetrate more easily through GI tract [30].

There are many methods for preparing CS nanoparticles such as emulsification and cross-linking, emulsion droplet coalescence, reverse micellisation [6]. In this study, we used emulsion solvent diffusion method with its simplicity and requirement of low energy sonication [15]. Honokiol, a potential anticancer drug, which has been reported recently to exhibit a potent cytotoxicity by inducing cell apoptosis in human fibrosarcoma cells, squamous lung cancer CH27 cells and SVR angiosarcoma cells [20-22], in rat and human leukemia cells [23, 24]. However, honokiol is water-insoluble and it was chosen as a drug loaded in PLGA nanoparticles which were modified surface by chitosan to form PLGA-CS nanoparticles to increase cytotoxic efficiency for tumor cells. Average size, zeta potential, encapsulation efficiency, morphology of this nanoparticles were investigated to indicate the role of each component, espe-

Volume : 5 | Issue : 2 | Feb 2015 | ISSN - 2249-555X

cially chitosan's position.

2.Materials and methods

2.1 Materials

Honokiol (98%) 10mg, PLGA with a lactide/glycolide molar ratio 50:50 (MW=7000-17000), low molecular weight chitosan (75-85% deacetylated, visocity 20-300 cP), polyvinyl alcohol (PVA, MW = 5000), and polyethylene glycol (PEG, MW= 5000), were purchased from Sigma-Aldrich (USA). Acetic acid was obtained from EMSURE (EMD Millipore Corporation, USA). Acetone was purchased from Shanghai (BiomanPharma Co. Ltd, Shanghai, China).

2.2. Methods

2.2.1. Preparation of PLGA-CS nanoparticles

The emulsion solvent diffusion method was conducted to synthesize PLGA-CS nanoparticles. PLGA and honokiol were dissolved into acetone to form oil phase fallen drop by drop into water phase which contained chitosan and surfactant compound dissolved in acetic acid 1% under stirring condition. As soon as oil phase diffused into water phase, nanoparticles were formed instantaneously.

Effect of each component to overall nanoparticle size and zeta potential was assayed. For each factor, various concentrations were used to evaluate the role of that factor as well as to find out optimized condition for formulation of PLGA-CS nanoparticles. After optimizing process, following condition was chosen: Oil phase contained 0.5 ml acetone dissolving 2.5 mg/ml PLGA and 1 mg honokiol. 0.1 % (w/v) chitosan and 5mg PEG were dissolved in 5ml acetic acid 1% to form water phase. Experiment was under 1000rpm stirring condition in 4 hours at room temperature. Synthesized nanoparticles were lyophilized and washed 3 times by ethanol.

2.2.2 Size average and zeta potential of nanoparticles

Size average of nanoparticles and zeta potential were identified by Zetasizer 3000 HS from Malvern instrument Ltd, UK.

2.2.3 Morphology

Morphology of honokiol nanoparticles was shown by scan electrical magnetic Hitachi S-4800 FE-SEM system, Japan.

2.2.4 Encapsulation efficiency

Nanoparticles (0.4 mg) were dissolved by 0.2 ml acetone. The mixture was vortexed in 5 minutes then transferred to hood cabinet. After acetone evaporated completely, 0.02 ml ethanol was added to collect honokiol. Concentration of honokiol in nanoparticles was measured by Nanodrop ND-1000 UV-Vis Spectrophotometer, Thermo Scientific, USA.

3 Results and discussion

3.1 Drug affected on size of nanoparticlesable 1. Effect of drug on average size of nanoparticles

	Oil phase			Water phase			Average size (nm)
	PLGA (mg)	Honokiol (mg)	Acetone (ml)	Chitosan (%)	PVA (mg)	Acetic acid 1% (ml)	
1st	10	1	0.4	0.02	50	2.4	539.5
2nd	10	0	0.4	0.02	50	2.4	400.3

As shown in table 1, in the same synthesized condition, presence of drug increased significantly nanoparticles size as well as slightly raised zeta potential. PLGA-CS nanoparticles increased in average size as compared with non-honokiol nanoparticles (539.5 nm in comparison with 400.3 nm, respectively). These nanoparticles were not suitable for oral administration due to their large size. Recent researches have indicated nanoparticles which are smaller than 500 nm cross easily M cells in GI tract [9] and stable nanoparticle system has to be higher 25 mV in zeta potential [8]. Therefore, concentration of other components as well as ratio between water phase and oil phase need to be adjusted to achieve higher nanoparticle quality.

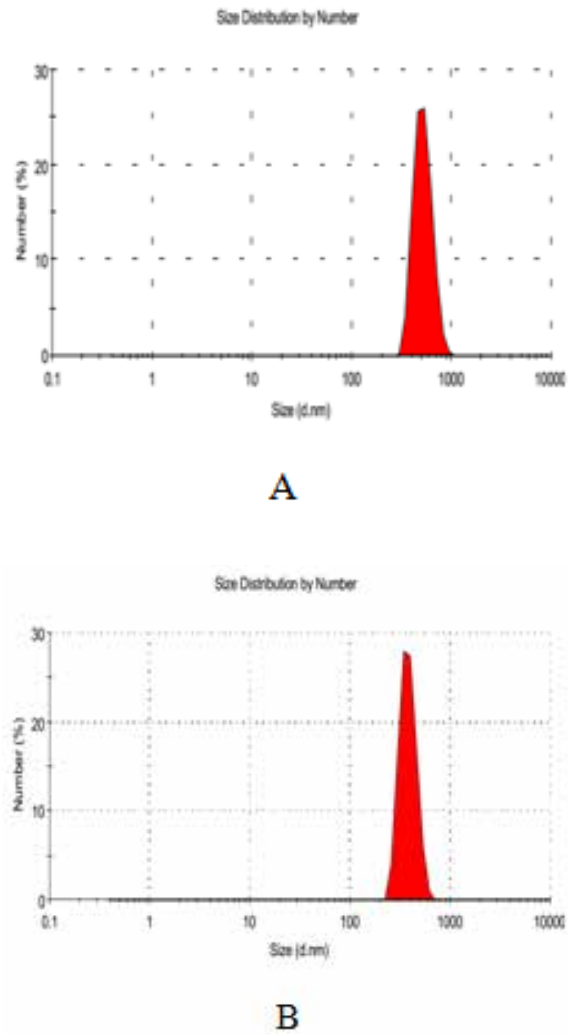


Figure 1. Average size of honokiol (A) and non-honokiol nanoparticles (B)

3.2.Effect of water/organic phase and surfactant on nanoparticle characteristics

The recent reports have showed that low water phase/organic phase ratio and high concentration of components have large effect in increasing average size of nanoparticles while zeta potential can be raised by adding higher concentration of chitosan [15, 17]. We adjusted components as in the following condition: 2.5 mg PLGA, 0.2 % (w/v) chitosan, water phase/ organic phase ratio was 10/1.

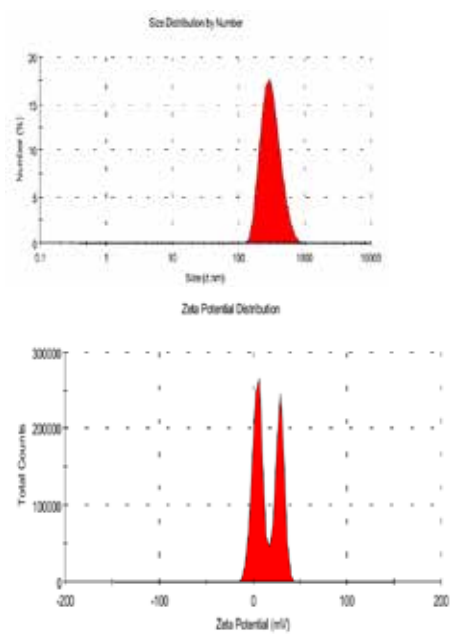
Volume : 5 | Issue : 2 | Feb 2015 | ISSN - 2249-555X

Figure 2a showed mean size of nanoparticles dropped from 400.3 nm to 342.3 nm and increase of zeta potential was observed when it reached 14.6 mV. However, presence of two peaks of zeta potential (4.61 and 27.8 mV) proclaimed that nanoparticle solution can have two nanoparticle types with different charge or it was heterogeneous surface charge system.

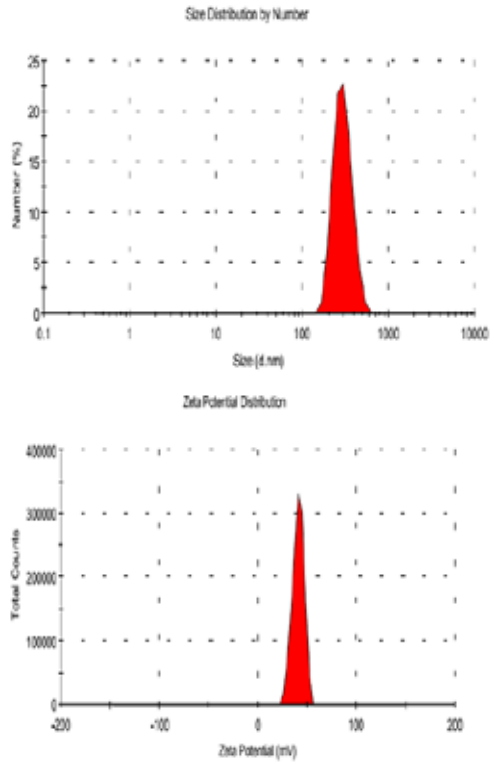
Data from table 2 showed that the use of PEG as surfactant instead of PVA are smaller in average size of nanoparticles with higher zeta potential. Nanoparticle solution with PEG-surfactant had average size of 307.0 nm and mA zeta potential of 40.7 mA while these parameters of nanoparticles with PVA-surfactant were 342.3 nm and 14.6 mA, respectively. However, the absence of surfactant produced white precipitation in solution. Thus, using PEG instead of PVA as surfactant resulted in smaller nanoparticles with higher homogenous. This result is similar to Castellanos et al. 2003 and Kumar et al. 2013, these researchers showed that PEG kept nanoparticles separated in solution better than PVA in the role of surfactant. Steric hindrance of PEG which is higher than PVA, reduces surface tension, result in its stronger dispersant characteristic which leads to smaller nanoparticles forming [25, 26].

	Organic phase		Water phase			Average size (nm)	Zeta potential (mV)
	PLGA (mg)	Acetone (ml)	Chitosan (%)	Surfactant (mg)	Acetic acid 1% (ml)		
1st	2.5	0.5	0.2	50 (PVA)	5	342.3	14.6
2nd	2.5	0.5	0.2	50 (PEG)	5	307.0	40.7

Table 2. Effect of different surfactants on average size and zeta potential of nanoparticles



A



B

Figure 2. Effect of different surfactants on mean size and zeta potential of nanoparticles. Nanoparticles with PVA (A) and nanoparticles with PEG (B)

Different concentrations of PEG were also tested. Due to high zeta potential of above obtained nanoparticles with PEG surfactant, 0.1 % chitosan was used to diminish size of PLGA-CS nanoparticles. In the same condition, as shown in table 3, reduced concentration of PEG led to slight change of average size and zeta potential. With 50mg PEG, nanoparticles had 268.8 nm average size, zeta potential reached 40.8 mV while average size and zeta potential of 5 mg PEG one after another were 246.3 nm and 41.3 mV. This result showed that, in comparison with 50 mg PEG, 5 mg of this component not only maintained itsimportant role in forming nanoparticles as well as high zeta potential but also reduced average size of PLGA-CS nanoparticles. We chose 5 mg PEG as optimized concentration of surfactant.

	Organic phase		Water phase			Average size (nm)	Zeta potential (mV)
	PLGA (mg)	Acetone (ml)	Chitosan (%)	PEG (mg)	Acetic acid 1% (ml)		
1st	2.5	0.5	0.2	50	5	268.8	40.8
2nd	2.5	0.5	0.2	5	5	246.3	41.3

Table 3. Effect of different concentration of PEG on average size and zeta potential of nanoparticles

3.3 Effect of chitosan

Results from table 4 indicated chitosan played a key role in providing positive charge on nanoparticle surface. As increasing concentration of chitosan, average size of nanoparticles was larger and zeta potential was higher, but

Volume : 5 | Issue : 2 | Feb 2015 | ISSN - 2249-555X

from 0.1% to 0.2% w/v concentration, while average size of nanoparticles increased, zeta potential was not different significantly.

Table 4. Effect of different chitosan concentration on average size and zeta potential of nanoparticles

	Organic phase		Water phase				
	PLGA (mg)	Acetone (ml)	Chitosan (%)	PEG (mg)	Acetic acid 1% (ml)	Average size (nm)	Zeta potential (mV)
1st	2.5	0.5	0%	5	5	200.7	26.5
2nd	2.5	0.5	0.1%	5	5	268.8	40.8
3rd	2.5	0.5	0.2%	5	5	307.0	40.7

3.4. Optimized formulation

Based on obtained results above, we decided the optimized condition to synthesize honokiol loaded PLGA-CS nanoparticles as following: 1 mg honokiol and PLGA 2.5 mg/ml were dissolved in 0.5 ml acetone. Chitosan 0.1% w/v, 5mg PEG dissolved in 5ml acetic acid 1%. Outcome as shown in Figure 4, nanoparticles achieved 43 mV zeta potential, average size was 253 nm with low PdI (0.07). PLGA-CS nanoparticles were sphere in shape by SEM images (Figure 5) and the efficiency of honokiol encapsulation was about 65%. PLGA nanoparticles without modified surface had low zeta potential [7] while our results showed that zeta potential of PLGA-CS NPs after optimizing was 43 mV and this value was suitable for stable delivery system [8]. Moreover, size of nanoparticles which are is than 500 nm in diameter is suitable for oral administration and they easily cross M cells in the Peyer's patches and the mesentery on the surface of GI mucosa to deliver drug into the systemic circulation [16].

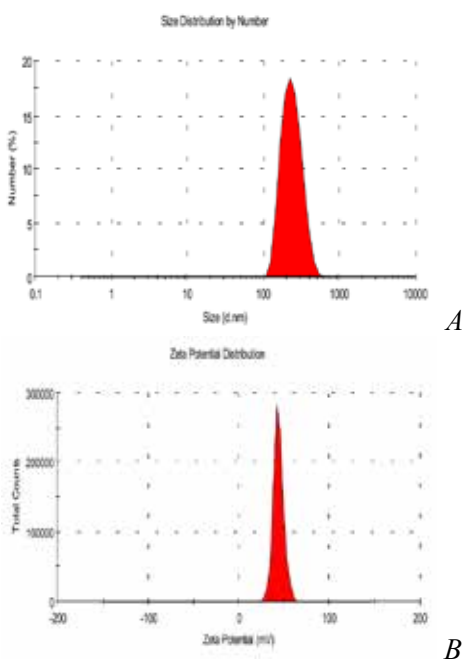


Figure 4. Size distribution (A) and zeta potential (B) of optimized nanoparticles.

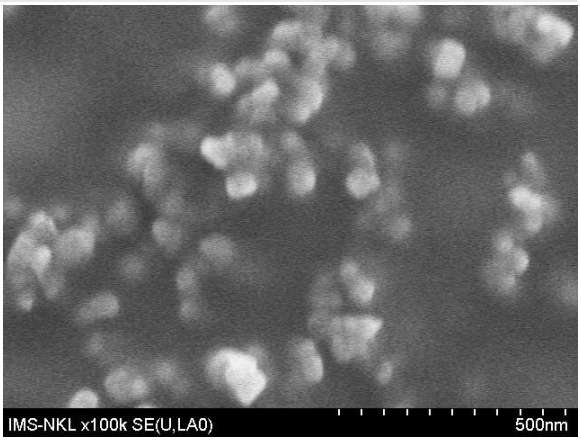


Figure 5. SEM image of PLGA-CS nanoparticles

Conclusion.

To sum up, honokiol loaded PLGA-chitosan nanoparticles quality depended on various factors which include concentration of drug, chitosan as well as presence of surfactant and water phase/organic phase ratio. We initially successed in optimizing conditions to synthesize honokiol loaded PLGA nanoparticles with modified surface by chitosan. Sphere PLGA-CS nanoparticles achieved stable quality of zeta potential and average size with high homogenous level for oral administration. Further researches need to be investigated to apply for anticancer medicine.

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Disclosure

The authors report no conflicts of interest.

Volume : 5 | Issue : 2 | Feb 2015 | ISSN - 2249-555X

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