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Study on characteristics, properties, and morphology of poly(lactic acid)/chitosan/hydroquinine green nanoparticles

<https://doi.org/10.1515/gps-2018-0025>

Received February 1, 2018; accepted July 16, 2018; previously published online August 15, 2018

Abstract: Poly(lactic acid)/chitosan (PLA/CS) green nanoparticles containing hydroquinine (Hq) were prepared by emulsion method. The content of Hq was 10–50 wt% compared with the weight total of PLA and CS. The characteristics of these nanoparticles were analyzed by Fourier transform infrared (FTIR), differential scanning calorimetry, field emission scanning electron microscopy (FESEM), and particle size analysis. The wavenumbers of C=O, C=N, OH, and CH₃ groups in FTIR spectra of the PLA/CS/Hq (PCHq) nanoparticles shifted in comparison with neat PLA, CS, and Hq that proved the interaction between these components. The FESEM images and particle size analysis results showed that the basic particle size of PCHq nanoparticles ranged between 100 and 200 nm. The Hq released from PLA/CS nanoparticles in pH 2 and pH 7.4 solutions was determined by ultraviolet-visible method. The obtained results indicated that the linear regression coefficient of calibration equation of Hq in the above solutions approximates 1. The Hq release from the PCHq nanoparticles includes fast release for the eight first testing hours, and then, controlled slow release. The Hq released process was obeyed according to the Korsmeyer-Peppas kinetic model.

Keywords: antimalarial; chitosan (CS); drug release; hydroquinine (Hq); nanoparticles; poly(lactic acid) (PLA).

1 Introduction

Malaria is known as the most common infectious disease caused by Plasmodium parasite. In 2015, risk of malaria was present in 91 countries. From 2010 to 2015, malaria incidence among populations at risk (the rate of new cases) decreased to 21% all over the world. Among all malaria-diseased age groups, about 35% were children under 5 years [1]. There were many different drugs used for the treatment of malaria such as artemisinin, chloroquine capsules, dihydroartemisinin-piperaquin tablet combination, artesunate, quinine drugs, etc. So far, quinine is still a valuable and effective drug in the treatment of malaria. It is said to be a highly effective antimalarial drug for the treatment of malaria [2]. Quinine and its derivatives metabolize in the liver and rapidly exhaust in the urine. The half-life elimination is about 11 h in a healthy person, but may take longer in malaria patients. The small amounts of quinine and its derivatives excrete through bile and saliva.

Recently, the biodegradable polymers were developed for use in different fields such as agriculture, forestry, food processing, and health. Poly(lactic acid) (PLA) is the most studied because of having many properties similar to thermoplastic polymers (polyethylene, polypropylene, and polyvinyl chloride) such as high tensile strength, high module, heat resistance, etc. [3]. In addition, the PLA also has the ability of combustion resistance, anti-ultraviolet radiation [4], especially the ability of biodegradation. PLA is considered as a versatile thermoplastic polymer and is increasingly used in engineering fields [5].

Chitosan (CS), a naturally occurring polymer, has also been extensively studied due to its superior features such as nontoxic, biodegradable, high antibacterial capacity, etc. [6]. It can be obtained by deacetylation of chitin that is found in many crustaceans such as crabs, lobsters, shrimp, etc. [7].

Combining the advantages of PLA and CS, nanocomposites based on PLA and CS are being increasingly studied. Due to good adhesion, biodegradability, and biodegradability, the PLA/CS (PC) nanocomposites are

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widely applied in drug delivery, systems surgical sutures, and tissue engineering [4, 8].

In this work, PC nanoparticles containing antimalarial drug – hydroquinine (Hq) were prepared by the emulsion method. These nanoparticles will be expected to treat the infectious malarial disease. Thanks to the reduction of the drug use dose and the drug use time. The Fourier transform infrared spectra (FTIR), particle size distribution, morphology, thermal properties of the PLA/CS/Hq (PCHq) nanoparticles, and *in vitro* release of Hq from the nanoparticles in different pH solutions were reported and discussed.

2 Materials and methods

2.1 Materials

Poly(lactic acid) (density 1.25 g/cm³, molecular weight 1.42×10^4 Da), CS (in powder, DD >77%, viscosity 1220 cPs), Hq (in white powder, purity ≥98%) were purchased from Sigma Aldrich (USA). Dichloromethane (DCM) and acetic acid were of analytical reagent grade and used without further purification were provided by Guangdong Guanghua Chemical Factory Co. (China).

2.2 Preparation

An aqueous solution of the Hq drug, Hq dissolved in ethanol was poured into the PLA solution using solvent DCM to form an emulsion of water/oil. Next, the emulsion mixture of water/oil was added into 1% acetic acid solution dissolved CS and polyethylene oxide (PEO) calculated by weight. The emulsion process was carried out for 15 min in a MAS-II microwave machine (Sineo Microwave, China). The PCHq nanoparticles were collected by centrifugation and then washed several times with distilled water in order to remove excessive PEO emulsifier before lyophilizing using a FreeZone 2.5 equipment (Labconco, USA). In this study, the PCHq nanoparticles samples were prepared at 10, 20, 30, and 50 wt% Hq (in comparison with PLA weight) and abbreviated as PCHq10, PCHq20, PCHq30, and PCHq50, respectively.

2.3 Characterization

The FTIR spectra of the PCHq nanoparticles were analyzed at room temperature by using the Nicolet/Nexus 670 spectrometer (USA). Each sample was recorded with 16 scans at a resolution of 4 cm⁻¹.

The size distribution of the PCHq nanoparticles was measured using a Zetasizer particle size analyzer (Malvern, England).

Thermal property of the PCHq nanoparticles was analyzed by using a differential scanning calorimetry (DSC-60) thermogravimetric analyzer (Shimadzu, Japan) from room temperature to 400°C at a heating rate of 10°C/min under argon atmosphere.

The morphology of the nanoparticles was observed on the FESEM images conducted using the S-4800 FESEM instrument (Hitachi, Japan). FESEM images were taken of sputtered samples with platinum coating.

The Hq released content from the nanoparticles in different pH solutions was calculated by UV-Vis spectroscopy method using a CINTRA 40, GBC spectrometer (USA).

3 Results and discussion

3.1 FTIR spectra

The FTIR spectra of Hq, PC, and PCHq nanoparticles are shown in Figure 1. In the FTIR spectrum of Hq, the characteristic band at 3178 cm⁻¹ can be assigned to –OH bending vibration. The peaks appeared at 2929, 1622, 1510, 1238, and 1033 cm⁻¹ corresponding to CH₃ group, aryl C=C and C=N– conjugated group, C–N amine group and C–O–C stretching vibration group, respectively. The FTIR spectra of PCHq nanoparticles indicated the characteristic peaks of the stretching vibrations of C=N, C–N, and C=C groups in Hq. In addition, the shift of wavenumbers of the groups such as C=O, C=N, C–N, C=C, C–O–C, –OH, –NH₂, and COOH in CS, PLA and Hq could be observed for the PCHq nanoparticles in comparison with the original PLA, CS, and Hq (Table 1). This could be explained by formation of hydrogen-bonding and dipole-dipole interactions between C=O group in PLA with OH and NH₂ groups in CS, and OH, C=N, and C–O groups in Hq.

3.2 The particle size distribution

The particle size distribution diagrams of PCHq nanoparticles using different Hq content (10, 20, 30, and 50 wt%) are presented in Figure 2.

Figure 1: Fourier transform infrared spectra of hydroquinine (Hq), poly(lactic acid)/chitosan (PLA/CS) (PC), and PLA/CS/hydroquinine (PCHq) nanoparticles.

Table 1: Wavenumbers of characteristic groups in Fourier transform infrared spectra of hydroquinine (Hq), and PLA/CS/hydroquinine (PCHq) nanoparticles.

Wavenumbers (cm ⁻¹)						
	Hq	PC	PCHq10	PCHq20	PCHq30	PCHq50
$\nu_{C=O}$	—	1754	1758	1759	1758	1759
ν_{CH_3}	2929	2993	2997	2944	2950	2948
ν_{CH}	—	2939	2952	3001	3000	2999
$\nu_{-OH, -NH_2}$	—	3421	3445	3433	3507	3451
$\nu_{C=C}$	1622	—	1625	1635	1634	1631
ν_{CN}	1510	—	1456	1458	1458	1456
ν_{C-O-C}	—	1183	1189	1189	1189	1190
	1033	1084	1094	1091	1091	1096

Figure 2: Particle size distribution diagrams of the PCHq nanoparticles using different Hq content.

It is clear that the particle size of PCHq nanoparticles ranged from 115 to 200 nm. The average particle size of PCHq nanoparticles was smaller than that of the PC nanoparticles (without Hq). The change in the particle size distribution can be clarified by the hydrogen-bonding and dipole-dipole between NH_2 and OH groups in CS with $C=O$ group in PLA, and $C=N$, $C-O$, and OH groups in Hq. This confirmed that Hq was incorporated into polymeric nanoparticles [4, 7]. The average particle size of the PCHq20 nanoparticle was smaller than that of other nanoparticles (Figure 2). This value of PCHq nanoparticles was smaller than that of PC nanoparticles loaded other drugs such as rifamicine, anthraquinone, and lamivudine [7, 9, 10]. The particle sizes of PC/rifamicine, PC/anthraquinone, and PC/lamivudine nanoparticles were 180–220, 100–200, and 300–350 nm, respectively.

3.3 DSC analysis

The thermal property of PCHq nanoparticles could be remarkably affected by the crystallization characteristics

Table 2: Differential scanning calorimetric data of poly(lactic acid) (PLA), chitosan (CS), and PCHq nanoparticles using different Hq content.

Sample	T_g (°C)	T_m (°C)	ΔH_m (J/g)	χ_c (%)
PLA	79.7	150.5	9.7	10.4
CS	110.7	—	—	—
PCHq10	65.1	154.4	18.9	20.3
PCHq20	68.6	153.2	22.6	24.7
PCHq30	64.9	152.4	19.0	20.4
PCHq50	65.2	154.4	20.4	21.9

χ_c (%) = $\Delta H_m \times 100 / \Delta H_m^*$ where ΔH_m^* is the heat of fusion for completely crystallized PLA (93.1 J/g); T_g , the glass transition temperature; T_m , the melting temperature; ΔH_c , the crystallization enthalpy; ΔH_m , the enthalpy of melting; χ_c , the degree of crystallinity.

of PLA and CS. The data of DSC analysis of PLA, CS, and PCHq nanoparticles using different Hq content are shown in Table 2.

From the DSC diagrams (Figure 3) and Table 2, it can be seen that neat PLA has a glass transition temperature (T_g) of 79.7°C, and a melting temperature (T_m) of 189°C. The T_g of CS is 110.7°C. The PCHq nanoparticles had T_g values between the T_g s of PLA and CS. The shift of T_g of PCHq nanoparticles in comparison with T_g of PLA and CS can be explained by the hydrogen-bonding and dipole-dipole interaction between OH, NH_2 , $C=O$, and $C=N$ groups in CS, PLA, and Hq as a rearrangement of the crystal structure of PLA. This displayed the simultaneous crystallization in PCHq and nanoparticles occurred due to the interactions as aforementioned. Thus, the degree of crystallinity (χ_c) of the PCHq nanoparticles was higher than that of neat PLA.

3.4 Morphology

The FESEM images of Hq and PCHq nanoparticles using different Hq content were expressed in Figure 4. It can be seen that Hq had an amorphous form and was irregularly sized, ranging between 1 and 5 μm (Figure 4A).

Figure 4(B–D) showed that the PCHq nanoparticles having a spherical shape with basic particle size was in the range 70–250 nm. The PCHq nanoparticle using 20 wt% Hq (PCHq20) had regular particle size and single dispersion. Its particle size was smaller than that of the nanoparticles using other Hq content (about 60–200 nm). However, all PCHq nanoparticles were agglomerated to form the particles with bigger size. The PCHq20 nanoparticle was less agglomerated than the other samples.

Figure 3: Differential scanning calorimetry diagrams of PLA, CS, and PCHq nanoparticles using different Hq content.

Figure 4: Field emission scanning electron microscopy images of Hq (A), PCHq nanoparticles using 20 wt% Hq (B), 30 wt% Hq (C), and 50 wt% Hq (D).

3.5 *In vitro* drug release

3.5.1 Determination of Hq drug loading efficiency from PCHq nanoparticles

The PCHq nanoparticles were dissolved in ethanol, then the Hq was released from the PCHq nanoparticles. The

released Hq content was determined by using UV-Vis spectroscopy method. Calibration equation of Hq dissolved in ethanol: $y = 6154x + 0.152$ [where x is the content of Hq (mol/l) and y is the absorption] with linear regression coefficient $R^2 = 0.991$. The Hq released content was calculated by the following equation: $\text{Hq (\%)} = \frac{m_t}{m_0} \times 100$ (where m_t is the amount of Hq released at time t , m_0 is

Figure 5: The absorbance versus different Hq content in pH 2.0 and pH 7.4 solutions.

the amount of initial Hq). The Hq released content from the PCHq10, PCHq20, PCHq30, and PCHq50 nanoparticles were 80.6, 84.4, 62.2, and 53.4 wt%, respectively. It is clear that the Hq released content was decreased with the rising initial Hq amount loaded to the PC nanoparticles. This can be explained by the agglomeration of Hq powder at high loaded Hq content which limit to add more Hq to the PC nanoparticles.

3.5.2 Setting up calibration equation of Hq in different pH solutions

The calibration equation of Hq in pH 2.0 solution and pH 7.4 solution were set up by using the UV-Vis spectroscopy method. Their linear regression coefficients were calculated according to the Excel software from the obtained data. The maximum wavelength of Hq in pH 2.0 and pH 7.4 solutions were 250.67 and 234.73 nm, respectively.

The calibration equation of Hq in pH 2.0 solution was $y = 37357x + 0.022$ with $R^2 = 0.997$ (approximate 1) showed a linear dependence of absorbance on the Hq content at $\lambda_{\max} = 250.67$ nm in the range of 3–12 g/ml (Figure 5A). Therefore, this wavelength was used to investigate the Hq

content released from the PCHq nanoparticles according to testing time (30 h).

Similarly, the calibration equation and the regression coefficient of Hq in pH 7.4 solution were displayed in Figure 5B. The calibration equation $y = 30556x + 0.059$ with $R^2 = 0.998$ indicated a linear dependence of absorbance on the Hq content at $\lambda_{\max} = 234.73$ nm in the range of 3–12 g/ml.

3.5.3 In vitro Hq release study

The Hq content released from the PCHq nanoparticles using different initial Hq content in pH 2.0 solution (corresponding to the portion of the stomach) and in pH 7.4 solution (corresponding to the duodenum) according to testing time (30 h) were determined by the UV-Vis spectroscopy method.

3.5.4 Effect of initial Hq content

The Hq released content from the PCHq nanoparticles using 10–50 wt% (comparison with the PLA weight) in pH 2.0 and pH 7.4 solutions was shown in Figure 6.

Figure 6: In vitro Hq released content from PCHq nanoparticles according to testing time.

The Hq content released from the PCHq nanoparticles included fast released period for the first testing time and then a controlled released period (slower release). The first fast released period occurred on the surface of the samples. The slower Hq release for the second testing period was started after eight testing hours because it took time for Hq to diffuse through the polymer matrix. It can be seen that the Hq released content from the PCHq nanoparticles in pH 7.4 solution was higher than that in pH 2.0 solution. This can be explained by: in pH 2.0 solution, the Hq released partially from the PCHq nanoparticles reacted with the acid solution to reduce the amount of Hq in the solution. This is consistent with the view in biomedical: Hq is poorly absorbed in the stomach, where its pH is small.

3.5.5 Release kinetic modeling

The HQ released kinetic study from the PCHq nanoparticles using 10–50 wt% of initial HQ content in pH 2.0 and pH 7.4 solutions was determined by different models such as zero order model (ZO), first order model (FO), Higuchi model (HG), Hixson-Crowell model (HCW), and Korsmeyer-Peppas model (KMP) [11].

The Hq released process from the PCHq nanoparticles using 10–50 wt% of initial Hq content in pH 7.4 solution

was carried out for testing 30 h according to various kinetic models as performed in Figure 7. Figure 7(A–D) indicated that the R^2 values of Hq released process from the nanoparticles according to FO, HG, and HCW models were 0.941, 0.901, 0.966, and 0.922, respectively. The highest R^2 value (0.979, Table 3) and all R^2 values in Table 4 belonged to the KMP model which was most suitable for reflecting Hq released process from the PCHq nanoparticles in pH 7.4 solution (Figure 7E).

The linear regression equations and the linear regression coefficient (R^2) of Hq released from the PCHq20 nanoparticles in pH 7.4 solution according to different kinetic models were presented in Table 3.

Similarly, the Hq released process from the PCHq nanoparticles using 10–50 wt% of initial Hq content in pH 2.0 solution was carried out in 30 h according to various kinetic models as shown in Table 5. The parameters of regression equations (R^2 and k) were calculated by using the different models (ZO, FO, HG, HCW, and KMP). The highest R^2 values (0.948–0.995) corresponding to the Korsmeyer-Peppas model also expressed this model was suitable for Hq released process from the PCHq nanoparticles in pH 2.0 solution.

Table 4 shows that the parameters of regression equation such as regression coefficient (R^2) and constant (k) that displayed the release process of Hq from PCHq

Figure 7: The Hq released kinetic from the PCHq20 nanoparticles in pH 7.4 solution [zero order model (A), first order model (B), Higuchi model (C), Hixson-Crowell model, and (D) Korsmeyer-Peppas model (E)].

Table 3: Regression equations and the regression coefficient (R^2) of Hq released from the PCHq20 nanoparticles in pH 7.4 solution according to different kinetic models.

Model	Regression equation	R^2
Zero order	$y = 0.557x + 28.58$	0.941
First order	$y = 0.010x + 1.584$	0.901
Higuchi	$y = 9.526 + 23.87$	0.966
Hixson-Crowell	$y = -0.031x + 1.282$	0.922
Korsmeyer-Peppas	$y = 0.269x - 1.184$	0.979

Table 4: Parameters of regression equation reflected Hq released process from the PCHq nanoparticles in pH 7.4 solution according to different kinetic models.

Model	PCHq10	PCHq20	PCHq30	PCHq50
ZO				
R^2	0.895	0.929	0.948	0.839
k	1.213	1.585	0.935	0.77
FO				
R^2	0.819	0.901	0.904	0.784
k	0.021	0.010	0.008	0.009
HG				
R^2	0.964	0.948	0.979	0.913
k	8.171	11.93	6.583	5.562
HCW				
R^2	0.847	0.922	0.921	0.803
k	-0.027	-0.031	-0.024	-0.023
KMP				
R^2	0.984	0.979	0.974	0.929
k	0.24	0.269	0.215	0.233

Table 5: Parameters of regression equation reflected Hq released process from the PCHq nanoparticles in pH 2 solution according to different kinetic models.

Model	PCHq10	PCHq20	PCHq30	PCHq50
ZO				
R^2	0.852	0.967	0.925	0.938
k	0.52	0.375	0.409	0.345
FO				
R^2	0.896	0.968	0.891	0.905
k	0.016	0.005	0.007	0.007
HG				
R^2	0.987	0.971	0.968	0.974
k	14.0	10.91	2.902	2.436
HCW				
R^2	0.912	0.969	0.903	0.917
k	-0.018	-0.013	-0.016	-0.015
KMP				
R^2	0.995	0.986	0.948	0.968
k	0.177	0.337	0.173	0.169

nanoparticles with different contents of Hq in pH = 7.4 are calculated based on different models (ZO, FO, HG, HCW, and KMP).

4 Conclusions

The FTIR spectra of Hq, PLA, CS, PC, and PCHq nanoparticles proved that Hq interacted with PLA, CS, and Hq was carried by the PC nanoparticles. The characteristic peaks of PCHq nanoparticles using different initial Hq content were shifted in comparison with the peaks of characteristic groups in original PLA, CS, and Hq. The degree of crystallinity in the PCHq nanoparticles was higher than that of neat PLA. The PCHq20 nanoparticle using 20 wt% Hq (PCHq20) had regular particle size and single dispersion. The Hq released process from the PCHq nanoparticles included fast released period for the first testing time and then a controlled slow released period. The Korsmeyers-Peppas kinetic model was the most suitable for Hq released study in pH 7.4 and pH 2.0 solutions.

Acknowledgments: The authors would like to thank the Vietnam Academy of Science and Technology for the financial support (subject code VAST.ĐLT.05/17-18, period of 2017–2018).

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