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PREPARATION AND CHARACTERIZATION OF CHITOSAN/ALGINATE/GINSENOSIDE Rb1 NANOPARTICLES

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Abstract. This work is focused on preparation and characterization of chitosan/alginate/ginsenoside Rb1 nanoparticles prepared by ionic gelation method with ginsenoside Rb1 content of 3, 5 and 7 wt.% in comparison with total weight of chitosan and alginate. Some methods including Infrared Spectroscopy (IR), Dynamic Light Scattering (DLS), Field Emission Scanning Electron Microscopy (FESEM) and Ultraviolet-Visible spectroscopy (UV-Vis) methods were used to determine the functional groups, size distribution, morphology and ginsenoside Rb1 release content from the chitosan/alginate/ginsenoside Rb1 nanoparticles, respectively. The ginsenoside Rb1 was dispersed regularly in chitosan/alginate matrix. The particle size of chitosan/alginate/ginsenoside Rb1 nanoparticles was reduced as increasing ginsenoside Rb1 content. The ginsenoside Rb1 release content from the chitosan/alginate/ginsenoside Rb1 nanoparticles in simulated body fluids was also evaluated and discussed.

Keywords: ginsenoside Rb1, functional groups, size distribution, drug release.

Classification numbers: 2.4.3, 2.7.1, 2.9.3.

1. INTRODUCTION

Ginsenoside Rb1 is a part of a class of steroid glycosides, and triterpene saponins. It is one of most abundant saponins in *Panax pseudo ginseng* possessing various useful bioactivities such as inhibition of the growth of cancer cells, reduction of cholesterols in blood, promotion of blood circulation and wound healing, etc. Moreover, it can affect on the reproductive system in animal testicles and increase testosterone production in male rats [1]. The ginsenoside Rb1 is poorly soluble in aqueous media and can be absorbed slowly into human's body with maximum concentration in plasma of 1090.45 ng/mL after 9.33 hours of administration [1 - 4]. In recent years, the use of biopolymers for loading ginsenosides to improve its solubility and bioavailability has been the area of intensive investigation [5 - 9]. Chitosan and alginate are two popular biopolymers with many advantages such as biocompatibility and non-toxicity [10 - 12]. These biopolymers have been applied for loading many drugs in microsphere and nanosphere

sizes, such as lovastatin, rifampicin, nifedipine, 5-fluorouracil, curcumin diethyl disuccinate, insulin, ampicillin, curcumin, verapamil, trimetazidine, etc. [13 - 16]. However, the study on combination of chitosan and alginate for loading ginsenoside Rb1 in nanoparticles has been still limited.

In our previous studies, chitosan/alginate/ginsenoside Rb1 or alginate/chitosan/lovastatin /ginsenoside Rb1 was prepared in composite films by solution method [17 - 19]. The obtained results show that the solubility of ginsenoside Rb1 can be improved through the load of chitosan/alginate matrix. However, crosslinking agent for forming these composite film systems has not been used yet.

In this work, the chitosan/alginate/ginsenoside Rb1 nanoparticles were prepared by ionic gelation method in the presence of sodiumtripolyphosphate (STPP) as a crosslinking agent and calcium chloride ($CaCl_2$) as a gelling agent. The characteristics including functional groups, particles size, morphology and ginsenoside Rb1 release control ability were evaluated and discussed.

2. EXPERIMENTAL

2.1. Materials

Alginate (AG, in powder, viscosity of 300 - 500 mpa.s), chitosan (CS, in powder, deacetylation degree > 77 %, viscosity of 1220 cP) and sodiumtripolyphosphate (STPP, in powder, purity > 98 %) were purchased from Sigma-Aldrich (USA). Ginsenoside Rb1 (in powder, purity > 99 %) was provided from China. Some other chemicals are commercial products of Viet Nam.

2.2. Preparation of chitosan/alginate/ginsenoside Rb1 nanoparticles

The chitosan/alginate/ginsenoside Rb1 nanoparticles were prepared by ionic gelation method using STPP crosslinking and $CaCl_2$ gelling agents. Firstly, various sample sollutions were prepared as follows: chitosan was dissolved in 30 mL of 1 % acetic acid solution (solution A), alginate was dissolved in 30 mL of distilled water (solution B), ginsenoside Rb1 was dissolved in 10 mL of ethanol solvent (solution C), STPP was dissolved in 2 mL of distilled water (solution D) and $CaCl_2$ was dissolved in 15 mL of distilled water to form 0.002 M solution (solution E). Secondly, 7.5 mL of solution E was added into solution D before being dropped slowly into solution C and the solution was magnetically stirred until it reached homogenous form (solution F). Next, solution F was dropped slowly into solution B which was being ultrasonicated continously (solution G).

Table 1. Weight of CS, AG, ginsenoside Rb1, STPP and design of CAR-NPs samples.

No.	Chitosan (g)	Alginate (g)	Ginsenoside Rb1 (g)	STPP (g)	Abbreviation of sample
1	0.1	0.1	0	0.02	CAR0
2	0.1	0.1	0.006	0.02	CAR3
3	0.1	0.1	0.010	0.02	CAR5
4	0.1	0.1	0.014	0.02	CAR7

Then, the solution A and 7.5 mL of solution E were introduced slowly into the solution G. The mixture was ultrasonicated and centrifugated at 4 ^{o}C to obtain the solid part. This part was lyophilized on FreeZone 2.5 machine to remove solvent completely. The CAR-NPs was obtained in light yellow powder after rubbed by agate mortar. The weight of component and design of CAR-NPs samples were presented in Table 1.

2.3. Methods of determination of characteristics

The functional groups of chitosan/alginate/ginsenoside Rb1 nanoparticles (CAR-NPs) were determined by infrared (IR) method on a Nicolet iS10 infrared spectrometer (USA). The IR spectra were recorded at room temperature in the wavenumbers of 4000 - 400 cm^{-1}, scans of 32 times and resolution of 8 cm^{-1}. The size distribution of CAR-NPs was determined by dynamic light scattering method on a Zetasizer (Malvern, UK). The samples were dispersed in distilled water before taking size distribution diagrams. The morphology of CAR-NPs was observed by Field Emission Scanning Electron Microscopy (FESEM) on a S4800 Hitachi (Japan) equipment.

2.4. *In-vitro* drug release study

0.2 g of CAR-NPs was added into 200 mL of buffer solutions (at pH 2 and pH 7.4 representing gastric and intestinal fluids in human's body, respectively. The solution was stirred on a magnetic stirrer at 37 ^{o}C for 32 hours. At interval time, 5 mL of solution was withdrawn, and 5 mL of fresh buffer solution was introduced into the solution to maintain the volume. From the calibration equations of ginsenoside Rb1 in pH 7.4 solution ($y = 17529x + 0.0479$, $R^2 = 0.9911$, $\lambda_{max} = 212$ nm) and pH 2 solution ($y = 28426x + 0.0124$, $R^2 = 0.9970$, $\lambda_{max} = 219$ nm) (in which y is optical density or absorbance of solution, x (mol/l) is concentration of ginsenoside Rb1 in solution and R^2 is linear regression coefficient), the concentration of ginsenoside Rb1 in solution at t time was calculated. The percentage of ginsenoside Rb1 released from CAR-NPs was calculated by dividing of the mass of drug at t time by the mass of drug at initial time and multiplying the result by 100.

3. RESULTS AND DISCUSSION

3.1. Functional groups of chitosan/alginate/ginsenoside Rb1 nanoparticles

The IR spectra of CAR-NPs prepared at different ginsenoside Rb1 contents are shown in Figure 1. The main functional groups of ginsenoside Rb1 are O-H, C-O, C-C, C-H. They are similar to functional groups of chitosan (O-H, C-O, C-H, C-C, N-H) and alginate (O-H, C-O, C-C, C-H, C=O), therefore, there are no new absorption peaks on the IR spectra of CAR-NPs (CAR3, CAR5, CAR7 samples) when introducing ginsenoside Rb1 into chitosan/alginate matrix as compared with those of the CAR0 sample. The wavenumbers of peaks characterized for C-O, C-C, C-H groups is negligible shift while the wavenumbers of O-H group vibration were changed of about 45 - 56 cm^{-1}. This means that ginsenoside Rb1 can interact with chitosan and alginate through hydrogen bonding of O-H groups. The formation of hydrogen bonding between drug and polymer matrix has been also proposed by Nafisa Gull *et al.* [20].

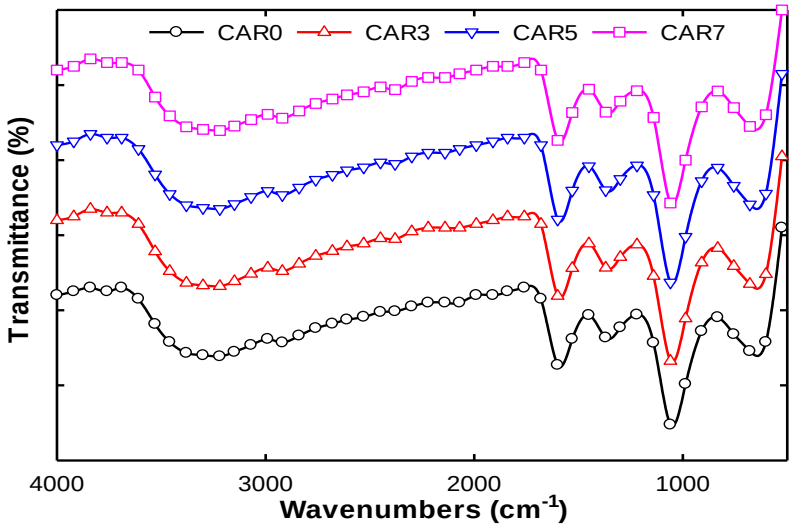

Figure 1. IR spectra of CAR-NPs.

3.2. Size distribution of chitosan/alginate/ginsenoside Rb1 nanoparticles

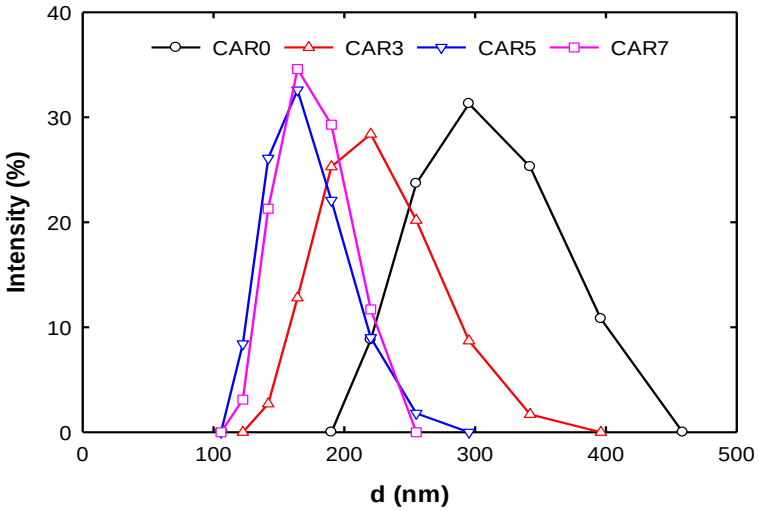

Figure 2. Particle size distribution diagrams of CAR-NPs.

From Figure 2, the range of size distribution of CAR0 sample is from 220.2 to 396.1 nm with average particle size of 301.8 ± 24.95 nm. The CAR3, CAR5 and CAR7 samples have smaller particle size, with the size distribution ranging from 122.4 to 295.3 nm.

As increasing the ginsenoside Rb1 content, the average particle size of CAR3, CAR5 and CAR7 samples is decreased. For example, the average particle size of CAR3, CAR5 and CAR7 samples is 228.1 ± 17.78 nm, 176.4 ± 12.30 nm, 172.3 ± 12.84 nm, respectively. This may be due to the fact that ginsenoside Rb1 was dispersed well in chitosan/alginate matrix and ginsenoside Rb1 can play a role of particle stabilizer [18], leading to the reduction of size particle of chitosan/alginate nanoparticles in the presence of ginsenoside Rb1 at suitable content. In comparison with other chitosan/alginate/drug systems [14 - 16, 21 - 22], the particles size of

CAR-NPs in this study is quite small (Table 2). This can be explained by the difference of nature of ginsenoside Rb1 and other drugs.

Table 2. Weight of CS, AG, ginsenoside Rb1, STPP and design of CAR-NPs samples.

No.	Sample	Average particle size	Ref.
1	Alginate/ chitosan microparticles loading insulin	7.5 μm	14
2	Alginate-reinforced chitosan nanoparticles loading rabeprazole	120 nm	15
3	Alginate coated chitosan core-shell nanoparticles loading naringenin	150 - 300 nm	16
4	Sodium alginate/ chitosan microparticles loading rifampicin	550 - 650 μm	21
5	Chitosan-reinforced alginate microparticles loading tegafur	146.3 μm	22

3.3. Morphology of chitosan/alginate/ginsenoside Rb1 nanoparticles

FESEM images of CAR-NPs prepared with different ginsenoside Rb1 content are presented in Figure 3. The CAR-NPs have a rough surface and heterogeneous structure with polymer matrix phase and ginsenoside Rb1 dispersion phase. The ginsenoside Rb1 was dispersed more regularly in chitosan/alginate matrix with size of 50 - 100 nm. Partial collapsing of the polymer network during lyophilizing process formed the polymer layers [23, 24]. The phase separation of alginate and chitosan was visually absent due to their good compatibility because of polyelectrolyte complex formation through $–NH_3^+$ of chitosan and $–COO^-$ of alginate [14, 24].

3.4. Drug release study

The ginsenoside Rb1 content released from CAR-NPs in pH 2 and pH 7.4 solutions is shown in Figures 4 and 5, respectively.

The drug release process commenced with a rapid release stage for the 10 first hours of testing, followed by a slower release stage in the remaining experiment period. In pH 2 solution, after first 10 hours, the ginsenoside Rb1 contents released from the CAR3, CAR5 and CAR7 samples reached 79.52 %, 90.37 %, 75.66 %, respectively. For following hours, the drug release content increased about 10 % for all tested samples. The same trend is also observed for ginsenoside Rb1 release process from CAR-NPs in pH 7.4 solution. Specifically, for 10 first hours, ginsenoside Rb1 was released from the CAR3, CAR5 and CAR7 samples with content of 80.78 %, 85.19 %, 82.34 %, respectively. As compared with ginsenoside Rb1 control sample, the drug release content in pH 2 solution from control sample is similar to that from the CAR-NPs while the ginsenoside Rb1 release from the CAR-NPs differs significantly with that from control sample in fast release stage in pH 7.4 solution. This suggests the drug release was controlled thanks to the biopolymer carriers and the hydrogen bonding between drug and polymer [20]. Current result agrees with the nifedipine release from alginate-chitosan coated beads in pH 7.4-buffer phosphate solution [23]. Therefore, the CAR-NPs are suitable for control ginsenoside Rb1 release in the intestinal tract.

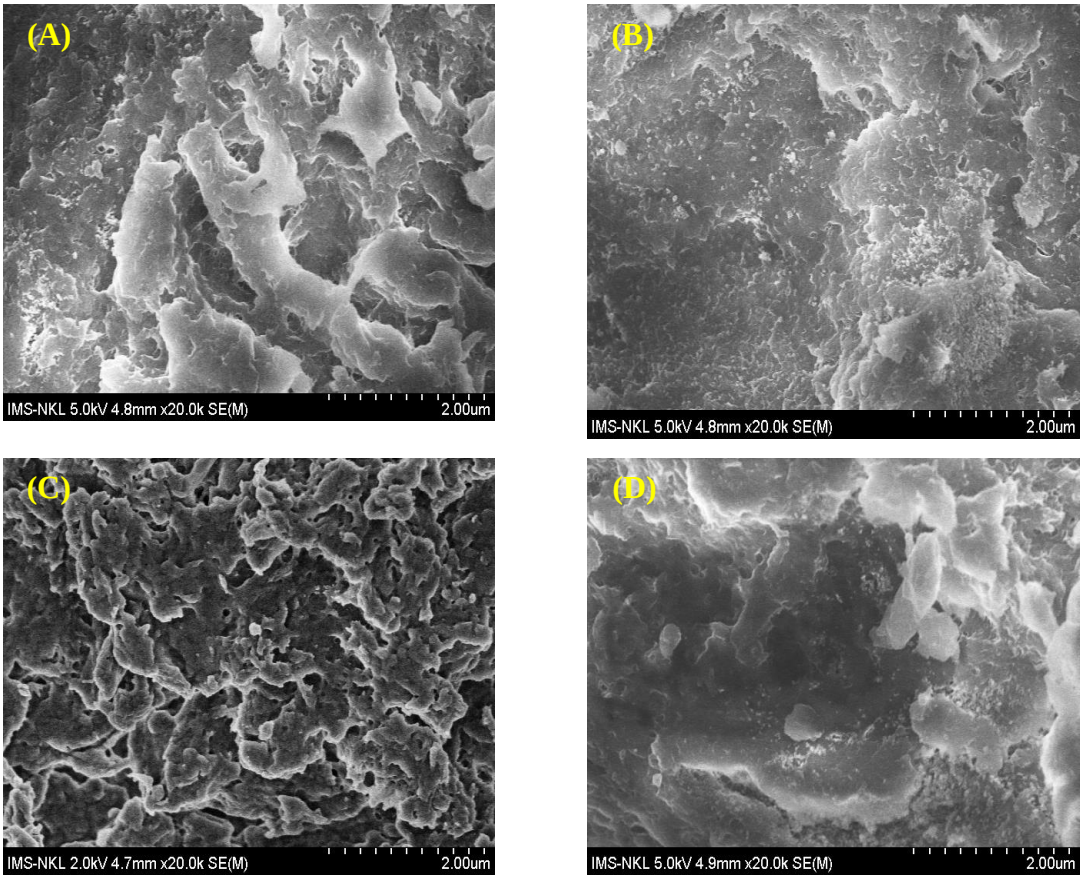

Figure 3. FESEM images of CAR-NPs, CAR0 (A), CAR3 (B), CAR5 (C), CAR7 (D).

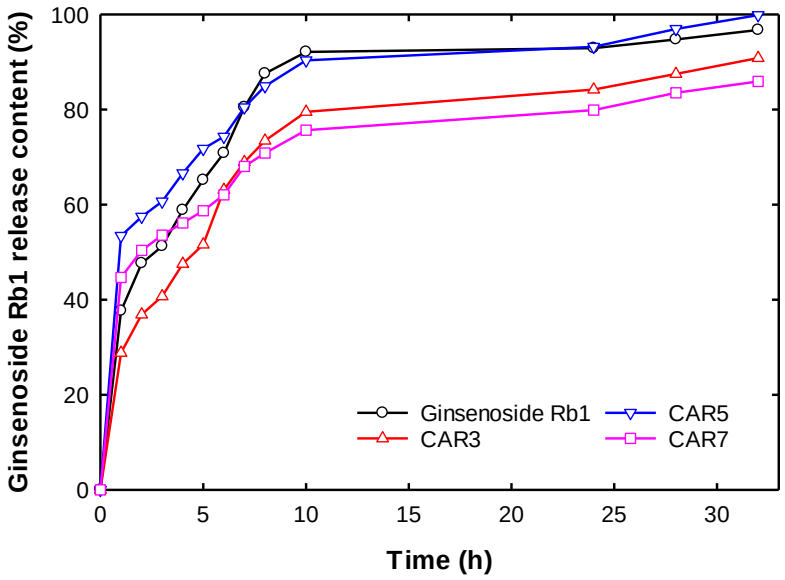

Figure 4. Graph of ginsenoside Rb1 release content from CAR-NPs in pH 2 solution.

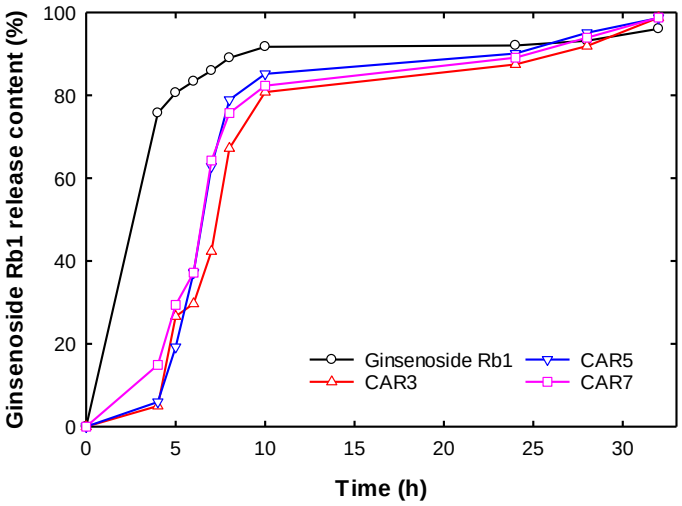

Figure 5. Graph of ginsenoside Rb1 release content from CAR-NPs in pH 7.4 solution.

Among tested samples, the CAR3-NPs exhibits a good control in drug release in both pH 2 and pH 7.4 solutions as compared with ginsenoside Rb1 control samples as well as other CAR-NPs samples. Therefore, 3 wt.% of ginsenoside Rb1 is the most suitable content to reach good effectiveness in drug release control by alginate/chitosan nanoparticles.

Study on the ginsenoside Rb1 release mechanism based on some popular kinetic equations such as zero order kinetic, first order kinetic, Hixson –Crowell, Higuchi and Korsmeyer-Peppas equations expressed that the fast release process of ginsenoside Rb1 in pH 2 solution follows to Hixson –Crowell model ($R^2 > 0.9799$) while the process of ginsenoside Rb1 release in pH 7.4 solution complies with Higuchi model ($R^2 > 0.9421$). The slow release process in both pH 2 and pH 7.4 solutions is most suitable with Korsmeyer-Peppas equation ($R^2 > 0.9779$) [14, 18]. This result also confirms that the release of ginsenoside Rb1 from the chitosan/alginate/ginsenoside Rb1 nanoparticles in pH 7.4 solution in fast release stage by diffusion mechanism of ginsenoside Rb1 through polymer matrix into solution.

4. CONCLUSION

In this work, the chitosan/alginate/ginsenoside Rb1 nanoparticles (CAR-NPs) were prepared successfully by ionic gelation method using STPP crosslinking and $CaCl_2$ gelling agents. The average particle size of the nanoparticles was 172.3 to 228.1 nm depending on the ginsenoside Rb1 content in the samples. The nanoparticles had a heterogeneous structure with rough surface. The ginsenoside Rb1 was dispersed regularly in polymer matrix. The result of ginsenoside Rb1 release from the CAR-NPs nanoparticles showed that the drug release process from CAR3 sample can be controlled in both pH 2 and pH 7.4 solutions. This indicates the CAR3 nanoparticles are promising candidate for delivery drugs in the gastrointestinal tract.

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Authors contributions: Thai Hoang (TH) had original scientific idea. TH and Nguyen Thuy Chinh (NTC) presented new point of this work. NTC and Pham Thi Hung (PTH) prepared the chitosan/alginate/

ginsenoside Rb1 nanoparticles. TH, NTC and PTH carried out analysis and assessment of morphology and properties of the nanoparticles. TH and NTC wrote the manuscript. All authors read and approved the final manuscript.

Conflict statement: There are no conflicts to declare.

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