Advanced Pharmaceutical Bulletin

Adv Pharm Bull, 2014, 4(Suppl 2), 549-554
doi: 10.5681/apb.2014.081
http://apb.tbzmed.ac.ir

Research Article

Formulation of Gammaoryzanol-Loaded Nanoparticles for Potential Application in Fortifying Food Products

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Article info

Article History:
Received: 11 July 2014
Revised: 3 August 2014
Accepted: 5 August 2014
ePublished: 31 December 2014

Keywords:
· Gammaoryzanol
· Nanoparticle
· Nutraceutical
· Antioxidant

Abstract

Purpose: The field of nanoparticle delivery systems for nutrients and nutraceuticals with poor water solubility has attracted a great attention during the last decades.

Methods: Ethyl cellulose (EC) based GO-loaded nanoparticles were prepared by solvent evaporation method. The effects of formulation parameters on nanoparticle size, encapsulation efficiency (EE%) and loading efficiency (LE%) were investigated. X-ray diffraction (XRD) and differential scanning calorimetry (DSC) techniques were used to investigate the crystalline behavior of GO and EC after the preparation of nanoparticles. Stability of the prepared nanoparticle was investigated during five weeks of storage.

Results: Particle sizes of all formulation were in the range of 70-100 nm with narrow size distribution. Increase in the time of sonication from 1 to 5 minutes decreased the particle size. However, the mean particle size was increased when the sonication time increased from 5 to 7 minutes. The results showed that in the same concentration of PVA, increasing the ratio of EC:GO led to an increase in the GO encapsulation efficiency and decrease in loading efficiency. During five weeks, the mean diameter and size distribution indexes (SPAN values) of nanoparticles did not show significant changes. DSC and XRD studies indicated that crystallinity of GO was decreased in nanoparticles.

Conclusion: EC based nanoparticles are promising carriers for addition of GO as a water insoluble antioxidant to fortify liquid food products without any change in quality of products.

Introduction

Nanotechnology has potential for numerous applications in different food industries.[1,2] Nanoencapsulation of bioactive components involves forming nanometric carriers with diameters ranging from 1 to 1000 nm.[3,4] Such systems can be potentially useful for controlled release applications in pharmaceutics, cosmetics and food industry, as well.[3-5] In the last years, the nanoparticle delivery systems have been increasingly employed in the case of nutrients and nutraceuticals with poor water solubility. Nanoparticle delivery systems have beneficial advantages such as increased surface area and reactivity, enhanced the solubility and bioavailability of nutraceutical compounds, especially poorly soluble substances such as functional lipids and natural antioxidants along with limited toxic effects.[6-8] Among the great variety of submicron particle, polymer-based nanoparticles are unique compared to other nanoparticle systems due to their better encapsulation, controlled release and less toxic properties.[9,10] Many processes have been developed for preparing polymeric nanoparticles including emulsification-solvent evaporation, emulsion polymerization, spray drying and interfacial polycondensation.[11-13] The emulsification-solvent evaporation method is based on the formation of an emulsion through the addition of a polymer solution to an aqueous phase followed by the removal of the solvent using evaporation to precipitate polymer as Nanospheres.[12,14] Nano precipitation method, developed by Thioune et al, produces polymeric based particles and is generally carried out by dissolving the core material in a fully or partly water-miscible solvent such as acetone or tetrahydrofuran and subsequently dropping the solution into an aqueous solution containing surfactant.[15,16] Numerous substances are known which can be used to entrap, coat, delivery and controlled release of bioactives in foods and nutraceuticals. Ethyl cellulose (EC) is a kind of semisynthetic modified cellulose, used for coating and controlled release applications. This derivative of cellulose is a biodegradable, biocompatible and hydrophobic polymer that has been reported to be advantageous as a carrier material to encapsulate an active agent by emulsion

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solvent evaporation technique,[17] coacervation phase separation technique, and spherical crystallization technique. It is also listed as GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA).[18] Oxidation reactions are happen when chemicals in the food exposed to the oxygen in the air. In natural conditions, animal and plant tissues contain their own antioxidants but in foods, these natural systems break down and oxidation is taken place. Food oxidation is a destructive process, causing loss of nutritional value and changes in chemical composition. In recent years, there has been a trend to use natural antioxidant compounds rather than synthetic antioxidants in the food industry.[19] Rice bran has been recognized as a rich source of several phytochemicals like oryzanols, tocopherols and vitamin E, which offer beneficial health properties and antioxidant activity.[20-22] Gammaoryzanol (GO) is a naturally occurring mixture of sterols and ferulic acid found in rice bran oil.[23] The potent antioxidant helps to stabilize vegetable oils and fats at elevated temperature,[24] improve the storage stability of foods[25,26] and increase its application in functional foods.[27] Other health beneficial properties of the antioxidants are improvement in plasma lipid composition as well as reduction of total plasma cholesterol and increase of HDL cholesterol levels and inhibition of the platelet aggregation.[28] However, incorporation of lipophilic antioxidant like GO in to liquid foodstuffs like beverages is limited due to its poor water solubility and changes in clarity of products. The purpose of this study was to produce a physicochemically stable nano particulate delivery system based on the EC, a biodegradable polymer, containing GO to improve the stability and functional properties of foodstuffs.

Materials and Methods

GO was received as a gift from Tsuno Rice Fine Chemicals Co., Ltd. (Wakayama, Japan). EC (45 cp) and polyvinyl alcohol (PVA) (27KD) were purchased from Sigma Aldrich (Steinheim, Germany). Distilled water was obtained from a Milli-Q Plus purification system and all other used chemicals and solvents were of analytical grade.

Preparation of polymeric nanoparticles

Polymeric nanoparticles were prepared by solvent evaporation method using EC as a polymeric carrier and GO as an active substance. The polymeric nanoparticles were prepared in three different ratios of EC:GO (1:2, 1:3 and 1:4) and different concentrations of PVA as surfactant (1%, 2% and 3%) by solvent evaporation method. GO and polymer were dissolved in 5 mL of ethyl acetate at room temperature using a magnetic stirrer. Ethyl acetate is a nonpolar organic solvent which is immiscible with water. Aqueous phase (20 mL) containing PVA was added into the organic phase dropwise using a syringe (22 G) connected to a programmable pump under high speed (20,000 rpm) homogenization (Heidolf, Germany) for 15 minutes followed by sonication with a high density ultrasonic probe (Sonicator Hielscher, UP200S, Germany) immersed directly into the solution kept in an ice bath. The organic solvent of fabricated nanoemulsion was removed by overnight evaporation at room temperature under continuous rotation to produce nanoparticles. The nanoparticle formulations with different concentration of PVA in aqueous phase and different ratios of EC:GO were summarized in Table 1. All formulations were prepared in triplicate.

Table 1. Formulation composition of Gammaoryzanol-loaded ethyl cellulose nanoparticles

Formulation Code	[a]EC:GO ratio (w/w)	[b]PVA (% w/w)
F1	3:1	1
F2	2:1	1
F3	4:1	1
F4	4:1	2
F5	3:1	2
F6	4:1	3

[a]Ethyl cellulose:Gammaoryzanol-loaded
[b]Polyvinyl alcohol

Particle size analysis

A laser light scattering particle size analyzer (SALD 2101, Shimadzu, Japan) was used to determine the particle size of the nanoparticles. Samples were suspended in distilled water and stirred continuously during the particle size analysis. The size and size distribution were expressed by the volume median diameter (VMD) and SPAN value, respectively. SPAN is a measure of the width of the size distribution calculated as the following equation:

$$SPAN = \frac{D_{(v,90)} - D_{(v,10)}}{D_{(v,50)}}$$

Where, D (v, 90), D (v, 10) and D (v, 50) are the equivalent volume diameters at 90, 10 and 50% cumulative volume, respectively.[29]

Encapsulation efficiency and loading efficiency

The encapsulation efficiency percent (EE%) and loading efficiency percent (LE%) of GO incorporated into the nanoparticles were calculated by using following equations:

$$EE\,(\%) = \frac{W_{(added\,GO)} - W_{(free\,GO)}}{W_{(added\,GO)}} \times 100$$

$$LE\,(\%) = \frac{W_{(added\,GO)} - W_{(free\,GO)}}{W_{(total\,polymer)}} \times 100$$

Where, $W_{(added\ GO)}$ is the amount of initial administered GO used for the preparation of nanoparticles, $W_{(free\ GO)}$ is the amount of unloaded free GO measured in the lower chamber of Millipore Amicon® after centrifugation, and $W_{(total\ polymer)}$ is the amount of used polymer in the preparation of nanoparticles. To separate unentrapped drug from nanoparticles, 1 mL of the

formulation was diluted with 4 mL of absolute ethanol (to dissolve possible non-entrapped GO) and then was placed into the upper chamber of Amicon® centrifugal filter (100K, Millipore, Billerica MA, USA) and centrifuged (Hettich EBA 20, Germany) at 21°C and 3000 rpm for 10 min. One mL of filtrate in the lower chamber of centrifugal filter was added to 5 mL of chloroform and the mixture was stirred at 500 rpm for 30 min. The amount of non-entrapped GO was determined by measuring the absorbance of GO in the sample collected from the lower chamber of filter spectrophotometrically at 319 nm (Shimadzu 8400 S, Japan).

Storage stability

Nanoparticle dispersions were stored at room temperature for a period of 35 days. Particle sizes were determined immediately after storage of samples at 25°C up to one month.

Differential scanning calorimetry (DSC)

A differential scanning calorimeter (DSC 60, Shimadzu, Japan) was used to determine enthalpy and melting point of all formulations. The equipment was calibrated using indium and zinc. Generally, 5 mg of the sample were weighed into an aluminum pan (40 micro liters), which was crimped non-hermetically, and heated in the range of 25–350°C at a scanning rate of 20°C/min. The melting points and enthalpies of fusion were calculated using the STARe software (version 8.01).

X-ray diffraction (XRD)

XRD analysis was performed using a nickel-filtered CuKα radiation (a voltage of 40 KV and a current of 20 mA) (Siemens D5000, Munich, Germany). The scanning rate was 2°/min over a 2θ range of 2-60° and with an interval of 0.02°.

Results and Discussion

Effect of formulation parameters on particle size

Average particle diameter of polymeric nanoparticles was determined by laser light scattering method. The particle size is an important parameter of formulation which can be influenced by its physicochemical properties. The particle size plays an important role in nanoparticle properties and hence a crucial task in characterization of nanoparticles is particle sizing. Lower particle size can be of great advantage because of the availability of large surface areas of the nanoparticles. It has been also shown that particle size is one of the important factors for the tissue and organ distribution of nanoparticles and several body distribution studies have shown that nanoparticles larger than 230 nm accumulate in the spleen due to the capillary size in this organ. On the other hand, different in vitro studies indicated that the particles size also influences the cellular uptake of nanoparticles.[30,31] Sonication time, as a process parameter, was assessed at the first step in order to achieve an optimal preparation conditions. The average diameter as a function of sonication time was shown in Table 2. As it was shown, increase in the time of sonication from 1 to 5 minutes led to the reduction in the particle size. However, the mean particle size was increased when the sonication time increased from 5 to 7 minutes. This might be attributed to the cavity formation and destruction caused by sonication which might induce agglomeration of nanoparticles in the liquid by increasing interactions and contact of nanoparticles.[32] Therefore, the optimum sonication time (5 minutes) was selected for the rest of the experiments.

Table 2. Effect of sonication time on the particle size (each number represents mean± standard deviation, n= 3)

Formulation code	Sonication time (min)	size (nm)
F1	1	232 ± 16
	3	186 ± 11
	5	94.5 ± 3
	7	301 ± 23

The effects of surfactant concentration and EC:GO ratio on the size and size distribution of polymeric based nanoparticles were illustrated in Table 3. Particle sizes of all formulation were in the range of 70 to 100 nm with narrow size distribution. Figure 1 shows the size pattern of formulation F5 extracted from particle sizer device in both volume (VMD) and number (NMD) mean diameters. The small difference between VMD and NMD indicates the homogeneity of the prepared nanoparticles.

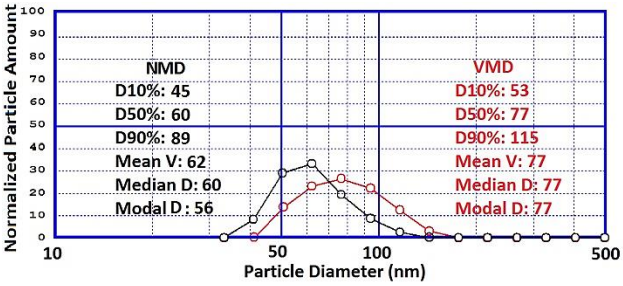

Figure 1. Particle size pattern of formulation F5 based on volume mean diameter (VMD) (red line) and number mean diameter (NMD) (black line).

Obtained results indicated that increase in the PVA concentration and EC:GO ratio did not change the particles size significantly. This may be due to the high impact of sonication on the particles size which undermined the effect of other variables in the studied range. However, the comparison of size distribution results indicated that increasing in polymer ratio resulted in an increase in particle size distribution. Particle size and size distribution is an important criteria of nanoparticles as these factors affect most of biological actions attributed to the size of particles such as drug release rate, biodistribution, mucoadhesion, and cellular uptake.[33] The microspheres with a narrow size distribution are necessary to optimize the clinical outcomes[34] but limited studies have been performed on biodegradable nanoparticles with narrow size

distribution. Therefore, assessment of the effect of process variables on size characteristics of nanoparticles in order to develop a method that can provide the uniform-sized nanoparticles composed of GRAS polymers was very imperative to us.[29,35] The nanoparticles are typically fabricated by high-speed homogenization or ultrasonication. In fact, the size of the nanoparticles prepared by these methods is difficult to be controlled and the size distribution is very broad. Consequently, poor reproducibility of the results may occur. For example, although many reports have shown that cellular uptake is associated with particle size, comparison of the data turned out to be confusing and even opposing due to broad size distribution.[36]

Table 3. Obtained experimental responses for different formulations (each number represents mean ± standard deviation, n= 3)

Formulation Code	Size (nm)	SPAN	[a]EE (%)	[b]LE (%)
F1	97.5 ± 9.79	1.24 ± 0.10	42.5 ± 7.19	18.2 ± 3.08
F2	94.5 ± 3.53	0.55 ± 0.64	51.6 ± 2.19	17.2 ± 0.73
F3	81.0 ± 4.24	0.88 ± 0.08	63.1 ± 2.74	15.7 ± 0.68
F4	91.5 ± 6.26	1.04 ± 0.04	65.3 ± 5.74	16.3 ± 1.43
F5	78.5 ± 0.71	0.82 ± 0.01	58.9 ± 8.98	19.6 ± 2.99
F6	82.0 ± 0.71	0.90 ± 0.06	77.9 ± 8.73	19.4 ± 2.18

[a]Encapsulation efficiency
[b]Loading efficiency

Effect of formulation parameters on EE and LE of GO

The effects of EC:GO ratio and surfactant concentration on GO encapsulation and loading efficiencies were assessed (Table 3). The results showed that in the same concentration of PVA, increasing the ratio of EC:GO led to an increase in the GO encapsulation efficiency and decrease in loading efficiency. Forming a thicker layer around the drug and thus preventing drug loss during preparation can be caused by higher polymer concentration.[37] On the other hand, increasing the PVA concentration increased both encapsulation and loading efficiencies of GO. This might be due to the stability of emulsion droplets caused by higher amounts of surfactant.[32]

Storage stability

Physical stability of GO-loaded nanoparticles was evaluated at room temperature over five weeks of storage. Particle size is an important feature regarding physical stability of nano formulations.[38] During five weeks, the mean diameter and SPAN value of nanoparticles did not show significant changes (Table 4) indicating that all of the formulations were stable as their sizes were below 100 nm over five weeks. The type of polymer used for preparation of nanoparticles and the suitable polymer:GO ratio might be the responsible for the observed physical stability of nanoparticles.

Table 4. Stability study of prepared nanoparticle (each number represents mean ± standard deviation, n=3)

Formulation code	Day 0		Day 21		Day 35	
	VMD (nm)	SPAN	VMD (nm)	SPAN	VMD (nm)	SPAN
F1	94.5 ± 3.53	0.55 ± 0.64	80.5 ± 0.70	0.85 ± 0.01	84.0± 7.07	0.98 ± 0.18
F2	97.5 ± 19.79	1.24 ± 0.10	89.5 ± 9.19	0.99 ± 0.21	88.5 ± 9.19	0.81 ± 0.06
F3	81.0 ± 4.24	0.88 ± 0.08	81.0 ± 7.07	0.97 ± 0.25	80.5 ± 0.07	0.50 ± 0.59
F4	91.5 ± 16.26	1.04 ± 0.04	83.0 ± 2.1	0.87 ± 0.00	83.5 ± 2.12	0.91 ± 0.02
F5	78.5 ± 0.71	0.82 ± 0.01	77.5 ± 0.07	0.83 ± 0.03	85.0 ± 9.89	0.97 ± 0.02
F6	82.0 ± 0.71	0.90 ± 0.06	81.5± 2.12	1.03 ± 0.23	74.5 ± 4.94	0.8 ± 0.03

Differential scanning calorimetry

DSC analysis was performed to investigate possible interactions between GO and polymer within the polymeric network of the nanoparticles and to define the physical state of the GO and polymer in the nanoparticles. Thermograms of GO loaded polymeric nanoparticle (Formulation F5), and the raw components individually (GO, EC and PVA) were displayed in Figure 2. The endothermic and exothermic peaks of GO and EC were observed at about 173°C and 160°C, respectively. The lack of endothermic melting peak of GO in the thermogram of nanoparticles (d) suggested that the encapsulation process produce a marked decrease in crystallinity of GO and changing to the amorphous state. The DSC profile of formulated GO nanoparticle showed an endothermic peak at 220°C which associated to the added PVA in the formulation. Therefore, for careful evaluation of the GO crystallinity status in the nanoparticles XRD experiments was done, as well.

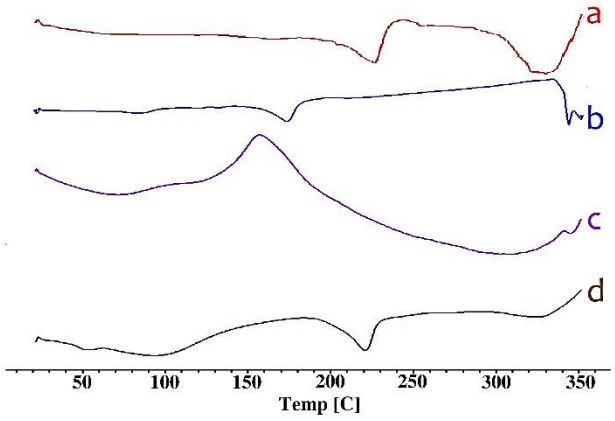

Figure 2. DSC thermograms of poly vinyl alcohol (a), gammaoryzanol (b), ethyl cellulose (c) and nanoparticles (F5) (d).

X-ray diffraction (XRD)

XRD diffractograms of GO, EC, PVA and nanoparticles (Formulation F5) were obtained to find out any change in the crystallinity of GO during encapsulation (Figure 3). The X-ray diffractograms of GO confirms its crystalline nature, as evidenced from the sharp and intense peak. XRD patterns of nanoparticle formulation exhibited a diffused peak that belongs to extra PVA. Absence of GO peak in XRD patterns of nanoparticle indicated to the change of crystalline nature of GO to the amorphous form. These results confirmed the results obtained from DSC analysis. Amorphous status of active ingredients offers several advantages. It is well known that amorphous compounds possess higher solubility, wettability (due to the lower contact angle of the drug particle surface with a liquid), dissolution rate, and bioavailability compared with crystalline materials.[39,40]

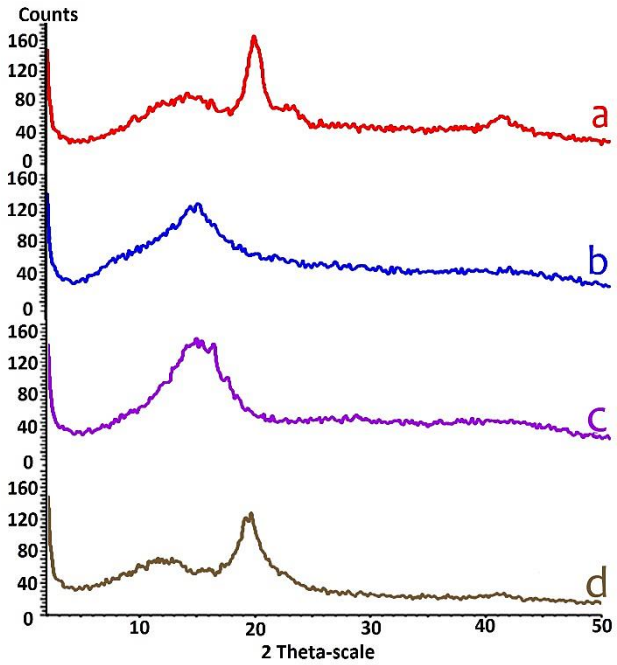

Figure 3. X-ray diffraction of poly vinyl alcohol (a), gammaoryzanol (b), ethyl cellulose (c) and nanoparticles (F5) (d).

Conclusion

The emulsion–solvent evaporation method allowed the preparation of nanoparticles using biodegradable and food grade polymer containing GO as nutraceutical. The GO encapsulation and loading were dependent to the concentration of surfactant and EC:GO ratio. However, these parameters did not affect particles size. DSC and XRD studies exhibited molecular dispersion of GO within nanoparticle. These nanoparticles, which had diameters of 70 to 100 nm, were physicochemically stable after 5 weeks. By using these stable GO loaded nanoparticles liquid food products, like beverages and nectars, could be fortified by a water insoluble antioxidant without any change in the quality, viscosity and clarity of food products.

Acknowledgments

We wish to thank the financial support of Drug Applied Research Center, Tabriz University of Medical Sciences.

Ethical Issues

Not applicable.

Conflict of Interest

The authors report no conflicts of interest.

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