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Formulation and optimization of Naringin loaded ß-lactoglobulin nanoparticles

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ABSTRACT

Protein derived from food-based nanoparticles are of great interest as they are recognized as safe and are easy to prepare. In this work we prepared ß- lactoglobulin nanoparticles for the improved bioavailability of naringin. The effect of pH, temperature, heating time and drug to protein ratio was optimized for particle size, PDI and encapsulation efficiency. The morphological features of the prepared particle revealed smooth spherical particles in clusters. The average particle size of the formulations ranged between 227 nm to 407 nm and the PDI ranged from 0.213 to 0.711. The zeta potential of the prepared ß-Lg nanoparticles was found in the range -4.1 to -7.4 mV suggestive to particle aggregation and the observed size range. The encapsulation of naringin was found in the range of 12.5% to 38.6%. The best formulation (F1) exhibited an encapsulation efficiency of 38.6% with particle size 227 nm and PDI 0.213. The in vitro release of F1 exhibited controlled release of naringin from the nanoparticles for 12 h. The results of the study led to the conclusion that protein nanoparticles (ß-lacotoglobulin) could be an effective method to improve bioavailability of phytomolecules.

Keywords: Naringin, ß-Lactoglobulin, nanoparticles, optimization, bioavailability

1. INTRODUCTION

Colloidal carriers have a double advantage in drug delivery system formulation: controlled release and the ability to target the drug to specific areas [1]. Over the years, poly(α-hydroxyacid)s such as poly L-lactic acid (PLA) have gained a lot of recognition as colloidal carriers. This is because (1) PLA is biodegradable under physiological conditions, which means its breakdown products are non-toxic and easily eliminated, and (2) manufacturing PLA allows for the control of nano-sized particles, which helps them evade the phagocytic system [2,3]. Potential targeting methods for site specific delivery are offered by the colloidal nanoparticles, which persist in circulation in high concentrations [4]. Nanoparticles are able to disperse throughout the body because their diminutive size means they can travel through the bloodstream more slowly and easily and because they can squeeze into tiny blood vessels called microcapillaries [5]. There are a lot of methods out there for making nanoparticles. Because to its simplicity and the excellent qualities of the formed particles, the solvent-evaporation technique has garnered special attention among the other methods [6].

The many desirable properties of food-based protein nanoparticles include their high level of controllability over size distributions, ease of manufacturing, and General Recognized As Safe (GRAS) status. Whey is a protein-rich by-product of cheese production that accounts for 85–95% of the original volume of processed milk [7]. The intriguing aggregation properties of beta-lactoglobulin (β-Lg), the main whey protein that makes up 51% (w/w) of total protein, have led to its utilization as an encapsulant and delivery vehicle. Modifying temperature, pH, and ionic strength can control the aggregation characteristics of β-Lg. A translucent "fine-stranded" gel is created when protein molecules are heated for an extended period of time in an environment with low pH and ionic strength. Within this gel, the protein molecules can form nanoparticles and assemble into long, rigid fibres [8]. According to the research, ß-Lg can be used to increase the bioavailability of hydrophobic compounds, as well as to dissolve and protect these molecules in water [9–17].

Phenolic compounds despite having have emerged as a class of natural products shown to have anti-oxidant, anti-atherogenic, and normolipidemic effects have limited use owing to poor aqueous solubility and low bioavailability. Naringin is a natural antioxidant isolated for *Grapefruit* and is known to possess a large number of pharmacological actions. The antioxidant potential of the molecule is mainly responsible for almost all of its effect on the human body. The half-life of the molecule is though low (3.5h) which limits the use of the molecule in therapy by lowering its bioavailability. Naringin is hydrolyzed to naringenin by gut flora prior to being absorbed and naringenin has been found

to have very low bioavailability of around 5.8% [18]. The aim of this work is to prepare beta lactoglobulin nanoparticles for the improved bioavailability of naringin.

2. MATERIAL AND METHODS

Naringin (>98%) was purchased from Yucca enterprises; ß-Lg was purchased from Sigma Aldrich; all other chemicals and reagents were purchased from CDH, Loba Chemie and Finar.

Preparation of ß-Lg nanoparticles

A 50 ml 0.2 % w/v β-Lg stock solution by dispersing the β-Lg powder in deionized water was made. It was then agitated the mixture magnetically for approximately 2 hours at room temperature. Overnight, at 4°C, this stock solution was allowed to fully hydrate in a 50 ml Falcon tube. After bringing the 5 ml sample from the β-Lg stock solution to room temperature and adding it to 15 ml of Falcon tube, the pH was determined. The γ-Lg dispersions were supplemented with Naringin in molar ratios of 5:1, 7.5:1, 10:1, and 12.5:1. Following this, the sample's pH was brought to 6.0 using 0.1M HCl/0.1M NaOH, with the exception of cases where the pH effect was examined (Table 1). Moving forward, a water bath that had been preheated to 75 °C was used to place the Falcon tube holding the sample. With the exception of investigations into the effect of temperature, the sample was left at this temperature for 45 minutes. The samples were incubated for the specified amount of time, then transferred to a cold bath for 10 minutes to end the process, and their pH was then measured [19].

Table 1. Batch formula for ß-Lg nanoparticles

Formulation	Drug (mg)	ß-Lg (mL)	Temperature (°C)	Time (min)	pH
F1	10	5	75	45	6
F2	10	5	75	45	5.7
F3	10	5	75	45	6.3
F4	10	5	60	45	6
F5	10	5	75	15	6
F6	10	5	75	30	6
F7	10	5	75	60	6
F8	15	5	75	45	6
F9	20	5	75	45	6
F10	25	5	75	45	6

Nanoparticle Characterization and Yield

By comparing the total mass of the drug and polymer utilized to make the nanoparticles to the mass of the finished product after lyophilization, the yield of the nanoparticles was arrived at.

Naringin calibration curve using UV

Naringin was most effectively absorbed in ethanol at a wavelength of 284 nm. At the aforementioned wavelength, the calibration curve was derived by experimenting with various medication concentrations. A freshly manufactured stock solution with a concentration of 100 µg/mL was made by dissolving 5 mg of Naringin in 50 ml of ethanol in a 10 ml volumetric flask. Then, the solution was filled up to the mark using the same buffer. To create standard dilutions with concentrations of 2, 4, 6, 8, and 10 µg/ml, portions of the stock solution were diluted with ethanol. A calibration curve was created by measuring the absorbance of each dilution at 284 nm using a UV spectrophotometer with ethanol as the reference blank [20].

Efficiency of Encapsulation [21]

Centrifugation was used for 30 minutes at 2000 rpm to remove naringin from the nanoparticle solution. The absorbance at 28 nm was measured using a UV spectrophotometer to examine the supernatant for free naringin. The calibration curve was used to determine the concentration of free naringin. This is the formula that was used to determine the encapsulation efficiency:

The equation for EE (%) is divided by the amount of naringin consumed, which is equal to the concentration of free naringin.

$$EE\ (\%) = \frac{concentration\ of\ free\ naringin}{amount\ of\ naringin\ used}$$

Surface shape

Scanning electron microscopy (SEM) was used to analyze the nanoparticles' surface topology and shape. Using a gold sputter module, a thin layer of gold-palladium was applied to the dried nanoparticles. A scanning acceleration voltage of

15 kV was applied to the coated sample.

Size, dispersion, and Zeta potential of particles

Using a Malvern Zeta sizer and the dynamic light scattering approach, we were able to estimate the average particle size, poly dispersity index (PDI), and Zeta potential of the nanoparticles. An examination was conducted at a 90° angle of detection using a disposable sized cuvette that contained ultrapure water suspended with the nanoparticles.

Naringin release from ß-Lg nanoparticles (*in vitro)*

Using a dialysis technique modified from Mishra et al [22], the naringin was released in vitro from the nanoparticles. After pre-swelling the dialysis membrane, nanoparticles loaded with naringin (1 mg) were distributed throughout 2 mL of phosphate buffer solution (PBS, pH 7.4). At 37°C, the membrane was placed on a biological shaker and submerged in 30 mL of PBS with a pH of 7.4. Fresh buffer was added to the same volume of fixed-volume aliquots of sample at predetermined intervals. Ultraviolet light with a wavelength of 284 nm was used to examine the partial volumes.

3. RESULTS AND DISCUSSION

In this study, nanoparticles of ß-Lg loaded with naringin were prepared and characterized. The effect of pH, incubation time, incubation temperature and molar ratio of drug to polymer was studied to obtain the most optimum formulation conditions. Particle size, PDI and encapsulation efficiency were observed as the dependent variables for selecting the optimized formulation. The ß-Lg solution was clear and transparent before pH adjustment and its original pH was 6.91. On adjusting the pH to the selected value (5.7-6.3) the solution turned turbid with higher turbidity at lower pH. This occurs due to aggregration of the ß-Lg suggesting the protein aggregation system on heating at high temperature.

Morphology, Particle size and PDI

The morphological features of the prepared particle revealed smooth spherical particles in clusters (Figure 1). The average particle size of the formulations ranged between 227 nm to 407 nm and the PDI ranged from 0.213 to 0.711. It was found that changing the pH above or below the isoelectric point of ß-Lg increased the particle size as well as PDI. Similarly increasing the drug to ß-Lg ratio increased the particle size and PDI. It was witnessed from the results that decreasing the heating time led to a slight decrease in PDI (Table 2).

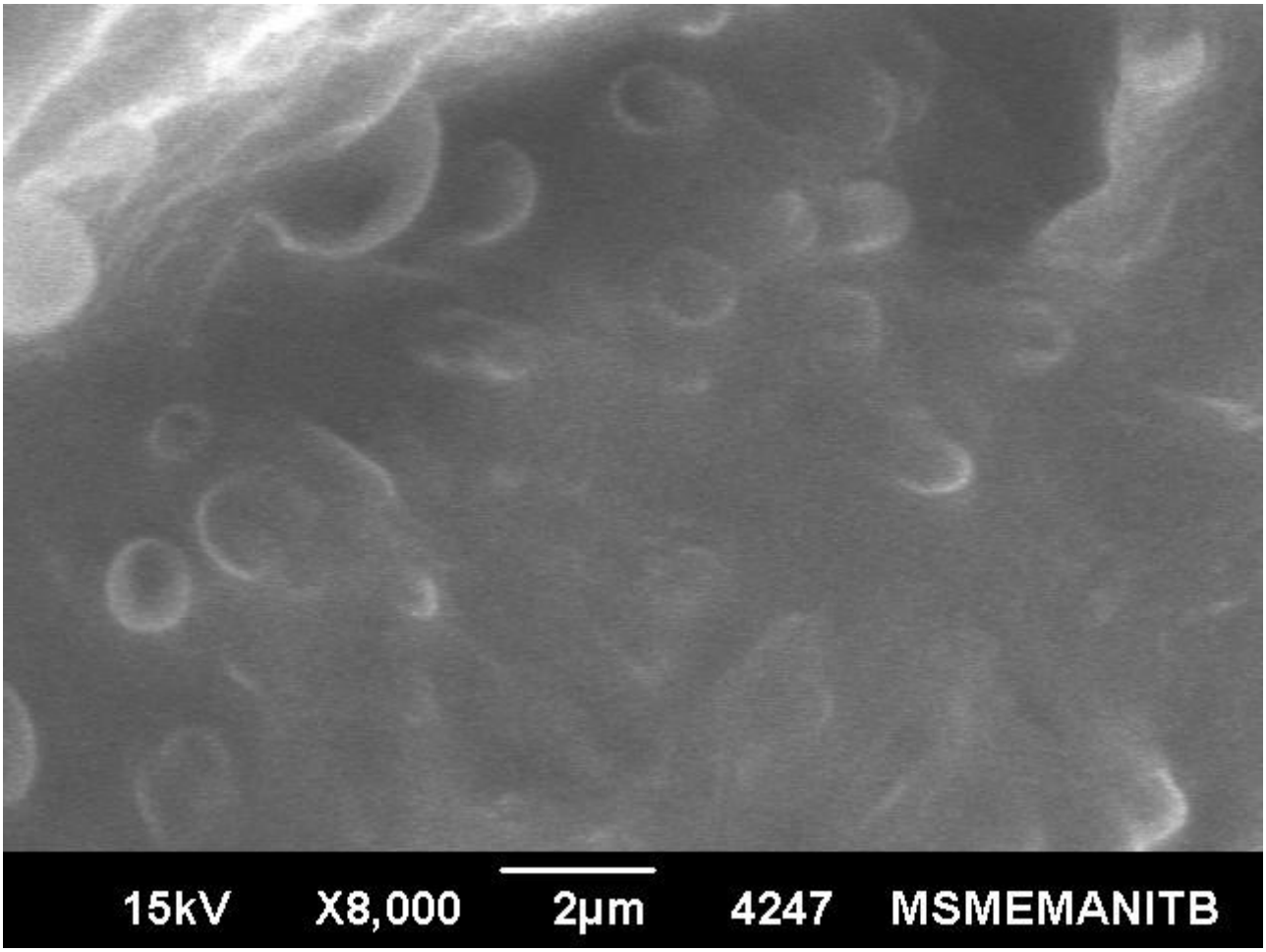

Figure 1. Surface pictograph of ß-Lg nanoparticles

Table 2. Evaluation results of prepared nanoparticles

Formulation	Size (nm)	PDI	Zeta potential (mV)	EE (%)
F1	227	0.213	-7.4	38.6
F2	245	0.417	-4.1	35.4
F3	253	0.418	-4.6	35.1
F4	301	0.429	-6.2	24.3
F5	313	0.371	-4.8	18.6
F6	311	0.348	-4.5	21.2
F7	353	0.621	-7.2	30.6
F8	375	0.618	-6.2	15.4
F9	382	0.625	-6.1	13.9
F10	407	0.711	-5.8	12.5

Zeta potential

The zeta potential of the prepared ß-Lg nanoparticles was found in the range -4.1 to -7.4 mV suggestive to particle aggregation and the observed size range. The zeta potential decreased in a steep manner when the pH of the system was reduced or increased. The decrease in heating time was also found to reduce the zeta potential (Table 2).

Encapsulation Efficiency

The encapsulation of naringin in the core of ß-Lg nanoparticles was calculated from the calibration curve of naringin (Figure 2) and was found in the range of 12.5% to 38.6%. The results revealed that the most critical factor affecting the encapsulation efficiency was the molar ratio of drug to protein. Increasing the ratio led to a drastic decrease in encapsulation efficiency (Table 2).

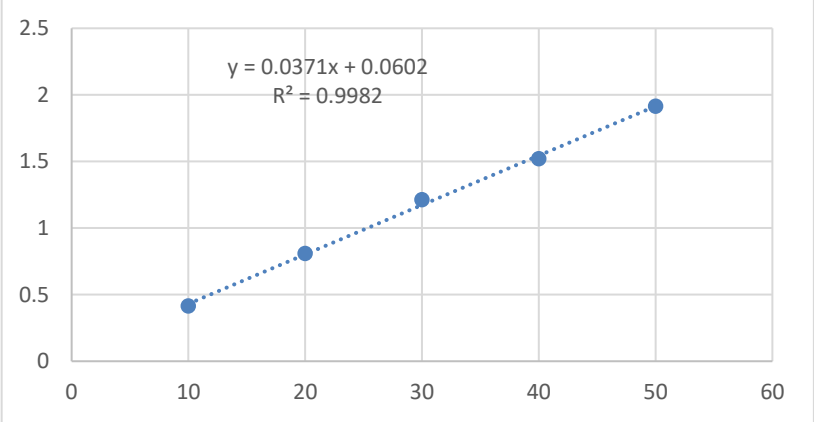

Figure 2. Calibration curve of naringin

In vitro release study

The calibration curve was used to calculate the concentration of naringin released from the nanoparticle. The nanoparticles released naringin steadily for upto 12 hours suggesting a controlled release (Figure 3). 98.7% of naringin was released at the end of 12th hour of the study.

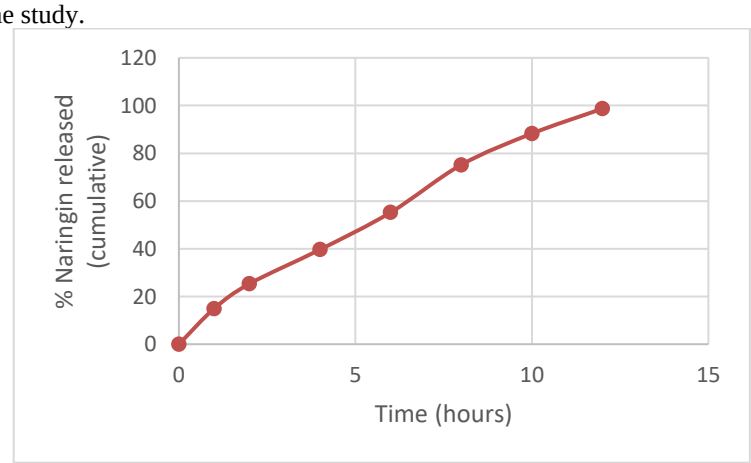

Figure 3. Cumulative release (*in vitro*) of naringin from F1

4. CONCLUSION

In this study ß-lactoglobulin nanoparticles loaded with naringin were optimized and characterized for their size, PDI, shape, encapsulation efficiency and *in vitro* release. The effect of pH, temperature, heating time and drug to protein ratio was optimized. Encapsulation efficiency, particle size and PDI were the dependent variables considered to optimize the formulation parameters. The best formulation (**F1**) exhibited an encapsulation efficiency of 38.6% with particle size 227 nm and PDI 0.213. The optimized conditions were found to be 5:1 molar ratio of drug to ß-Lg, 45 min heating at 75°C and pH of 6.0. The *in vitro* release of **F1** exhibited controlled release of naringin from the nanoparticles for 12 h. The results of the study led to the conclusion that protein nanoparticles (ß-lacotoglobulin) could be an effective method to improve bioavailability of phytomolecules.

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