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Fabrication And Characterization Of Curcumin And Spirulina Loaded Nanoparticles

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***Abstract*— We refer to the use of nanotechnology to make nano (Curcumin-Spirulina) with the carrier alginate - a polysachharide extracted from seaweed. With nanosize, Nano (Curcumin-Spirulina) product is expected to dissolve well in water, highly bioavailable and able to target by the passive mechanism, therefore, reduce the disadvantages and improve the health, prevention and treatment of cancer of Curcumin as well as Spirulina**

Keywords—Curcumin; Sprirulina, nano (Curcumin-Spirulina) product

I. INTRODUCTION

Spirulina belongs to the group of primitive microorganisms, called Cyanobacterium or Blue-green microalga. Spirulina is consumed in many countries because of its high nutritional value and health benefits. It is considered as a super food, a miracle of the sea. Once upon a time, Spirulina has been known to be a source of nutritious food from the Aztecs in Mexico and the Aboriginal people of Kanembu, Central Africa [1]. Up to this time, the nutritional value and biological activities of Spirulina have been widely distributed in many countries around the world such as USA, France, Japan, Canada, Mexico, Taiwan, and China... According to many studies, Spirulina is rich in protein, up to 60-70%, amino acids, especially non-substitution amino acids, rich in vitamins, minerals, pigments and many biologically active substances. [2]. In addition, its three main biologically active ingredients which are biliprotein pigment phycocyanin, sulfated polysaccharides and gamma linolenic acid play an important role in improving human body's function. Research results show that these compounds support immune system, promote health and have antiviral effects. [3].

Although many of the vitamins, amino acids, and proteins in Spirulina are good for health, they are hydrophobic, readily aggregated leading to be reduced in body absorption, or easily eliminated from the body. Therefore, the requirement set out for scientists is to apply technology in enhancing the efficacy of product.

Nanotechnology has recently emerged with many important roles in all aspects of life. In the pharmaceutical industry, nanotechnology is well known for its remarkable features, for example: : enhancing the durability of the active ingredients under the influence of environmental factors such as temperature, light, moisture, pH,..., improving absorption into the body, increasing the solubility and enhancing the effectiveness and reducing the side effects of the active ingredient [4]. In the world, many nano drug delivery systems, such as:Doxil®, AmBisome®, NanoTherm®, Marqibo®… have been studied and applied in treatment with high efficiency and few side effects [5]. Research team of Dr. Ha Phuong Thu at Institute of Materials Science, Vietnam Academy of Science and Technology has made many researches using nanotechnology to fabricate nano-Curcumin and obtained very positive results. Curcumin is an active substance extracted from turmeric and has many precious biological activities, for instance: antioxidant, anti-inflammatory, immune-enhancing, preventative and supportive treatment of many cancers [6]. However, curcumin has a limited to use as a drug to treat disease because of its poor solubility in water and its low oral bioavailability. Curcumin nanoparticle systems are made using biodegradable polymers that are less than 100 nanometers in size, improving the solubility and enhancing the effects of curcumin. These results have been published in many international journals [7-10]. Recently, nanocurcumin product combined with saponin from Panax Pseudo-Ginseng Wall and fucoidan have also been developed and demonstrated the effect by the research team and transferred to the pharmaceutical company to produce functional foods.

II. MATERIALS AND METHODS

A. General experiment procedures

a) Spirulina extract:

0.9 g of Spirulina algae biomass was dispersed in 50 ml of distilled water. The mixture was then ultrasonicated for 30 minutes to break down the cell

membrane and to release active ingredients in the algae cell. Next, the mixture was centrifuged at 5000 rpm for 5 minutes and the juice extract of Spirulina was obtained after removing insoluble ingredients.

b) Fabrication of Nano (Curcumin-Spirulina)

3.00 g of alginate was dissolved in 50 ml of water, then combined with the Spirulina extract prepared above. After that, a solution containing 0.6 g of Curcumin dissolved in 100 ml of ethanol was slowly added to the aqueous solution, under ultrasonication for uniform dispersion. The mixture was stirred overnight to complete the nanofabrication process. After evaporating completely the solvent,Nano (Curcumin-Spirulina) was obtained. In order to easily preserve the nanoparticles, we conducted freeze-drying to obtain Nano (Curcumin-Spirulina) in powder form.

B. Materials

Curcumin was purchased from Institute of Chemistry - Vietnam Academy of Science and Technology. *Spirulina platensis* was grown and harvested in Hue. Alginate and ethanol were purchased from Merck. Distilled water was used in all experiments.

C. Characterization of Nano (Curcumin-Spirulina) System

Surface morphology and particle size of materials were investigated by field emission scanning electron microscopy (FE-SEM) (Hitachi S-4800). Surface charge and particle size distribution were determined by zetasizer ZS 90. The material structure was determined by Fourier transform infrared spectroscopy (FT-IR) (SHIMADZU).

The content of Curcumin or Spirulina in the sample was quantified by measuring the UV absorption of the solution after dissolving the Nano (Curcumin-Spirulina) in the appropriate solvent and calculating the results based on the standard curve had been built.

III. Results and Discussion

A. Fouourier transform infrared spectroscopy (FT-IR)

From the FT-IR spectra (Fig. 1), we have fabricated Curcumin-Spirulina-encapsulated alginate nano system by combining the characteristic absorption peaks of functional groups present in the IR spectrum of the nanoparticles. The shift of these functional groups demonstrates the interaction in the nanosystems.

In the IR spectrum of the Nano (Curcumin-Spirulina) system: a wide absorption band of 3600-2500cm^{-1} belongs to the stretching vibration range of the -OH group of the -COOH group, the absorption peak at 1635 cm^{-1} belongs to stretching vibration of the C = O group (a strong shift compared to the original substances: 1628 in Curcumin and 1650 in Spirulina). The absorption peak of the C = C aromatic ring of Curcumin at 1512 cm^{-1} was shifted to 1519 cm^{-1} in the spectrum of the nano-system. Similarly, the absorption peaks of the C = C bond in Spirulina were also shifted from 1550 cm^{-1} to 1589 cm^{-1} in the spectrum of the final product. Bonding vibration of the C-O-C group of Spirulina was also shifted when incorporated in the nano-system: from 1080 transitions to 1142 cm^{-1}.

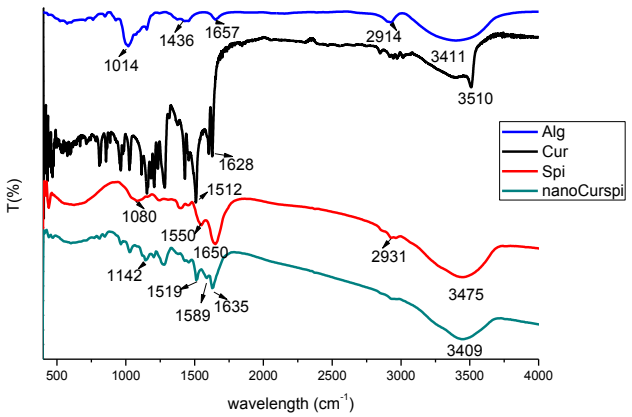

Fig 1. FT-IR spectra of curcumin, spirulina, alginate and Nano (Curcumin-Spirulina) system

These evidences show that Nano (Curcumin-Spirulina) system has been formed and there is interaction between the components.

B. FESEM image

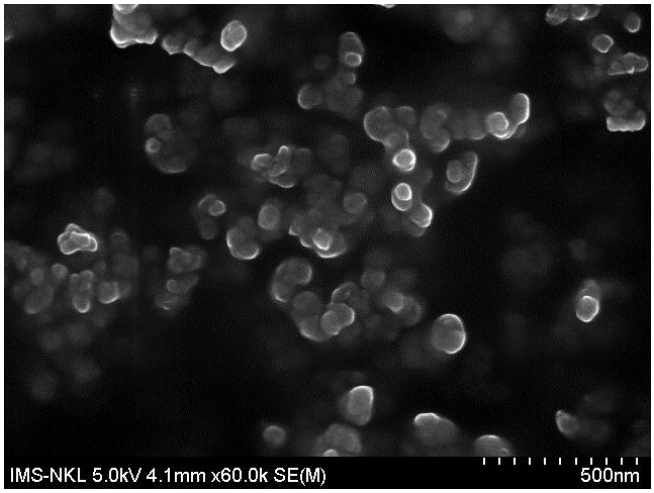

Fig 2. FESEM image of Nanoparticles (Curcumin-Spirulina)

From the FESEM image (Figure 2) we can see that the obtained nano systemare spherical, less aggregative, relatively uniform in size, mostly about 50-70 nm.

In theory, when introduced into the body, the nano drug delivery system are considered to be foreign bodies and will be identified and eliminated by the reticuloendothelial system (RES) before reaching the target. Therefore, particle size is an important factor in ensuring the duration of circulation and the effect of drug delivery system. Studies have shown that particles larger than 200 nm will be rapidly detected and excreted from the body by the immune system, while particles smaller than 5 nm will be easily excreted by the kidneys. [11]. In addition, the difference in vascular structure of tumors and blood

vessels of healthy tissue is a key factor in the ability to accumulate drugs in tumors in a passive targeting mechanism based on the EPR effect (Enhanced Permeability and Retention effect) [12]. Unlike healthy blood vessels with tight structures, tumor vasculature is leaky with the gaps as large as 600-800 nm. As a result, particles of less than 400 nm can easily penetrate the tumor without accumulating in healthy tissue. In addition, the dysfunction of the lymphatic system in the tumor also reduced the excretion of the particles from the tumor as compared to that in healthy tissue. This leads to prolonged the duration of circulation of drug at the cancerous tumor [13].

C. DLS and zeta potential

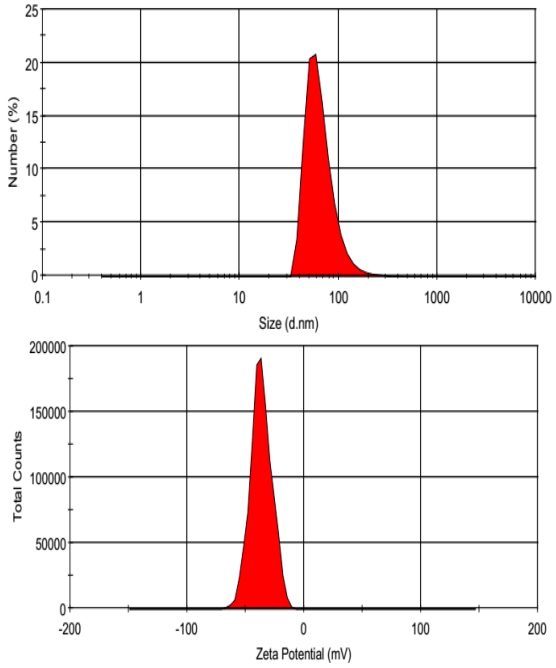

Fig 3. Size distribution and zeta potential of Nano (Curcumin-Spirulina) system

The size and size distribution of the system obtained by the DLS method is shown in *Figure 3*. Accordingly, the mean diameter of the seed is about 123.7 nm with a narrow size distribution (PDI = 0.242). The system is fairly uniform and is within the optimal size range. Compared with the size obtained from the SEM images, the particles in the solution were larger because of the interaction of alginate and spirulina composition with the water environment.The high stability of the system with highly negative zeta potential (-36.7 mV) gives a great value for use in biomedical applications.

In conclusion, with an average size of below 100 nm, the Nano (Curcumin-Spirulina) system is expected to be optimal size to achieve targeted effect in the passive mechanism, and not be quickly eliminated from the body.

D. Determination of Curcumin and Spirulina content

The content of Curcumin/Spirulina in the product was calculated by the formula:

$$H\% = \frac{\left(\frac{A-b}{a}\right)*k*V}{m} \quad (1)$$

where a, b are the coefficients of the standard curve equation; A is the maximum absorption of Curcumin/Spirulina in the solution after diluting k times from a solution containing m (mg) of the product dissolved in V ml of alcohol/water mixture (for Curcumin)/water (for Spirulina).

The content of Curcumin and Spirulina in the product has been calculated successfully as shown in *Table 1*. The results showed that alginate has a good loading capacity, with 12.46% Curcumin and 18.94% Spirulina were loaded.

Table 1. Content of components in the sample

	A	a	b	K	V	m	Content %
Curcumin	0.782	118.51	0.0436	20	11.5	11.5	12.46
Spirulina	0.0607	1.8916	0.0009	6	100	100	18.94

In this study, we have fabricated Curcumin and Spirulina-loaded nano alginate system with spherical shape, uniform distribution, with size about 100 nm. The system had a negative zeta potential of -36.7 mV. Curcumin content loaded in the nano system was 12.46%, Spirulina was 18.94%. Thus, Nano (Curcumin-Spirulina) system is considered to be the ideal candidate for combining Spirulina with Curcumin in a nano-system to have an effective effect on cancer cells. Further studies about drug release as well as *in vitro* and *in vivo* assays should be conducted to assess the activity of the product.

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