STABILITY OF 'SUBMICRON CURCUMIN' FROM *CURCUMA LONGA* L. IN AQUEOUS MEDIA

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(Manuscript Received on May 13th, 2011, Manuscript Revised November 01st, 2011)

ABSTRACT*: Curcuma Longa L. has been considered as an excellent source of curcumin, having wide scale applications in pharmaceutical, food and cosmetic industry. However, widespread applications of this relatively efficacious agent have been limited due to the poor aqueous solubility, and consequently, minimal systemic bioavailability. In this research, 'submicron curcumin' suspension in aqueous media was prepared from Curcuma longa L. without the presence of any additive. The average diameter of the 'submicron curcumin' particles was 546 nm (DLS). It was found that the 'submicron curcumin' suspension could be used in conjunction with several common food ingredients with its stability remaining unaffected. As the system only contained 'submicron curcumin' and water, it would be highly promising to applications in functional food, cosmetic, and pharmaceutical industries.*

Keywords: *Curcuma Longa L.*

1. INTRODUCTION

Curcumin has attracted a considerable attention in recent years due to its great variety of beneficial biological and pharmacological activities [1]. Besides its effective antioxidant, antitumor, anti-inflammatory, anticarcinogenic and free radical scavenger properties, it is also believed that curcumin is a potent agent against many diseases such as anorexia, rheumatism, and Alzheimer disease [2]. However, widespread clinical application of this relatively efficacious agent in cancer and other diseases has been limited due to poor aqueous solubility, and consequently, minimal systemic bioavailability [3]. Attempts to improve water solubility, stability, and bioavailability of curcumin by complex formation or interaction with macromolecules including gelatins, polysaccharides and phospholipids were previously reported [2,3]. However, *toxic organic solvents* were still present in these procedures.

As curcumin is used in foods and traditional medicines, the development of effective approaches to improve the solubility and stability of curcumin in *aqueous media* is significantly necessary. Recently, nanoparticle technology has emerged as a potential area of targeted drug delivery systems and made biologically availability of therapeutic agent [4]. Nanoparticles and submicron particles are believed to pass through cell membranes in organisms and get rapid interaction with biological systems. A highly efficient method to prepare 'submicron curcumin' (diameter < 1μm) from *Curcuma longa* L. using nanoparticle technology, without the presence of any additive was recently reported [5]. In this paper, we wish to report the stability of the as-prepared 'submicron curcumin' in aqueous media.

2. EXPERIMENTAL

2.1. Preparation of 'submicron curcumin'

Chemicals were purchased from Sigma-Aldrich and Merck, and used as received without further purification. Powdered rhizomes of turmeric (*Curcuma longa* L.) were extracted with ethanol at 40 ^oC and at the solid / liquid ratio of 1/7 (g / ml) for 3 hours, according to slightly modified previous procedures. The mixture was then filtered under vacuum to remove the solid residue. High pressure steam from an autoclave was injected into the curcumin solution to remove the ethanol and the turmeric essential oil present in the solution. Turmeric resin adhered to the flask bottom during the steam injection procedure. The suspension of 'submicron curcumin', being produced during the steam procedure, was cooled to 10 ^oC to separate and remove oleoresin and conventional curcumin powder.

2.2. Characterization of 'submicron curcumin'

Curcumin content was determined by UV-VIS (ultraviolet-visible) spectroscopy method using standard solutions of curcumin. The curcumin isolated from *Curcuma longa* L. was also analyzed by HPLC-MS (high-performance liquid chromatography-mass spectrometry). The turmeric essential oil separated from the procedure described above was analyzed by GC-MS (gas chromatography-mass spectrometry) at HCMC Center of Analytical Services & Experimentation. The particle size distribution of curcumin suspension was determined by dynamic laser light scattering (DLS) method using a LA 920. Transmission electron microscope (TEM) studies were performed using a JEOL JEM 1400, in which samples were dispersed on holey carbon grids for TEM observation.

2.3. Stability of 'submicron curcumin' in aqueous media

As the 'submicron curcumin' is designed for application in food technology, we intended to investigate its stability in aqueous media at 10 ^oC in the presence of some common ingredients as mentioned below:

- Preservatives including methyl paraben at concentrations of 0.01%, 0.02%, 0.03%, 0.04%, and 0.05%; propyl paraben at concentrations of 0.01%, 0.02%, 0.03%, 0.04%, and 0.05%; potassium sorbate at concentrations of 0.05%, 0.1%, 0.15%, 0.2%, 0.25%, and 0.3%; and sodium sorbate at concentrations of 0.05%, 0.1%, 0.15%, 0.2%, 0.25%, and 0.3%, respectively.
- Sweeteners including saccharose at concentrations of 10%, 20%, 30%, 40%, 50%, and 60%; honey at concentrations of 10%, 20%, 30%, 40%, 50%, and 60%, respectively.
- Heavy metal cations including Fe^{2+} at concentrations of 0.1 ppm, 0.2 ppm, 0.3 ppm,

0.4 ppm, and 0.5 ppm; Cu^{2+} at concentrations of 0.125 ppm, 0.25 ppm, 0.5 ppm, 0.75 ppm, and 1 ppm, respectively.

The 'submicron curcumin' suspension was dissolved in ethanol and the curcumin content was determined using UV-VIS spectroscopy. The stability of curcumin was defined as percentage of total concentration of the remaining curcumin compared to the initial amount of curcumin: S(%) = (A_t/A_0) x 100 (%), where A was the absorbance of the curcumin solution in ethanol /water.

3. RESULTS AND DISCUSSION

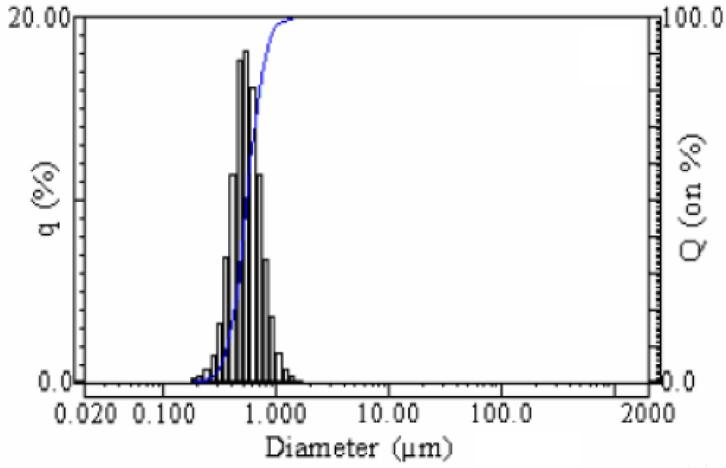

Figure 1. Particle size distribution of the 'submicron curcumin' (DLS)

A mixture of curcumin, oleoresin, and essential oil from *Curcuma longa* L. was extracted into 98% ethanol, according to slightly modified previous procedures. Under this condition, curcumin was achieved in 97% yield (analyzed by HPLC-MS and UV-VIS) compared to total curcumin obtained using soxhlet extraction method. It should be noted that essential oil and oleoresin were also recovered as by-products in the procedure, with 4.5 % essential oil and 9% turmeric resin being achieved (compared to total dry turmeric powder). The curcumin solution in ethanol was then used to prepare 'submicron curcumin' as previously reported [5]. Steam from an autoclave at 1 atm was used in the procedure. Particle size distribution of 'submicron curcumin' suspension in water was measured using TEM and DLS methods. The yield of 'submicron was determined, comparing to the total curcumin dissolved in the starting ethanol solution used in the procedure.

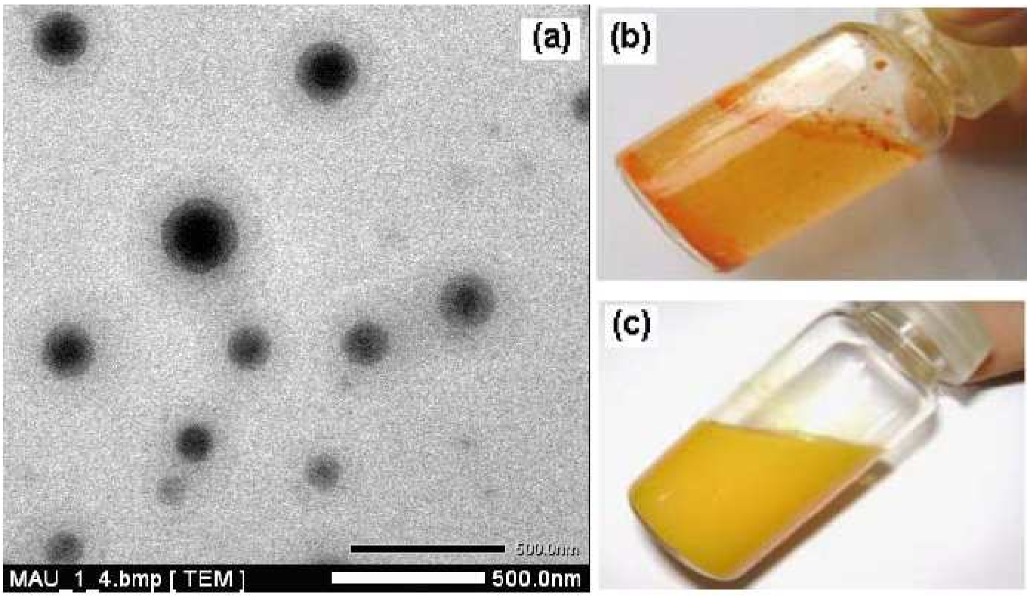

Figure 2. TEM micrograph of 'submicron curcumin' (a), pictures of conventional curcumin powder (b) and 'submicron curcumin' (c) dispersed in water.

Using the procedure described in the experimental section, a 'submicron curcumin' yield of 92.5% was achieved as compared to the total amount of starting curcumin. The actual concentration of curcumin solution was measured by UV-VIS method using standard solutions of curcumin. DLS analysis showed that the average diameter of the 'submicron curcumin' was 546 nm (Figure 1). TEM micrograph of the 'submicron curcumin' indicated that the particle size of the 'submicron curcumin' was approximately 200 nm (Figure 2a). However, the TEM image exhibited only a limit of particle size range, while the DLS result reflected a full particle size distribution in the suspension. It was observed that conventional powder curcumin was poorly dispersible in water (Figure 2b), while the 'submicron curcumin' was fully dispersible in water (Figure 2c). It should also be noted that the λ_{max} in the UV-VIS spectrum of curcumin solution in ethanol was identical to that of 'submicron curcumin' suspension in water (Figure 3).

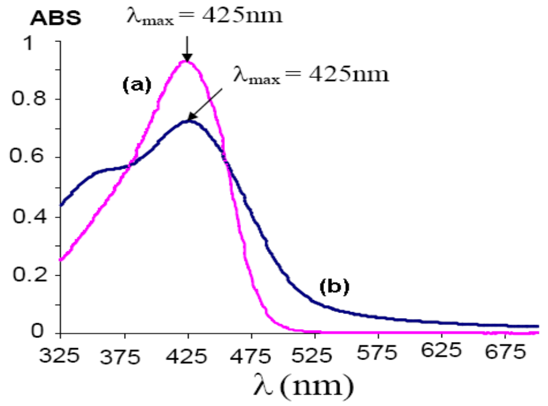

Figure 3. UV-VIS spectra of curcumin in ethanol (a) and 'submicron curcumin' in water (b)

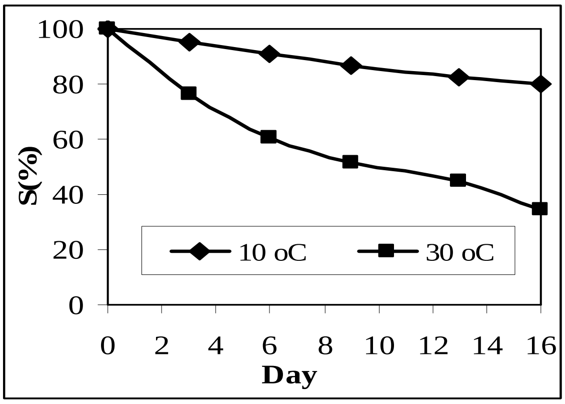

Figure 4. Effect of temperature on the stability of 'submicron curcumin'

The stability of 'submicron curcumin' in aqueous media was then investigated within 16 days. Initial research addressed the effect of temperature, having carried out the experiment at room temperature (approximately 30 ^{o}C). It was observed that the stability of 'submicron curcumin' decreased significantly under this condition, with 52% and 35% curcumin remaining in the suspension after 8 days and 16 days, respectively. As the 'submicron curcumin' is designed for application in food technology, we also performed the experiment at 10 ^{o}C, which is the normal temperature in a conventional fridge. Experimental results showed that the stability of 'submicron curcumin' could be improved at this temperature as compared to that at room temperature, with 79% curcumin being achieved after 16 days (Figure 4). It was therefore decided to store the 'submicron curcumin' suspension at 10 ^{o}C in a conventional fridge for further research.

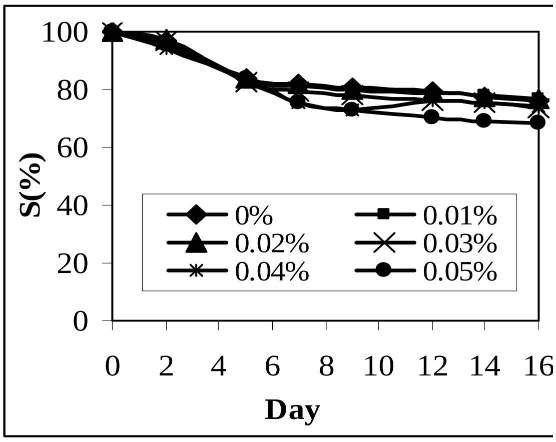

Figure 5. Effect of methyl paraben concentration on the stability of 'submicron curcumin'

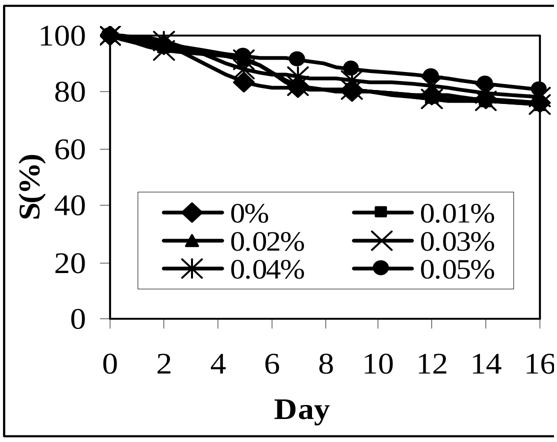

Figure 6. Effect of propyl paraben concentration on the stability of 'submicron curcumin'

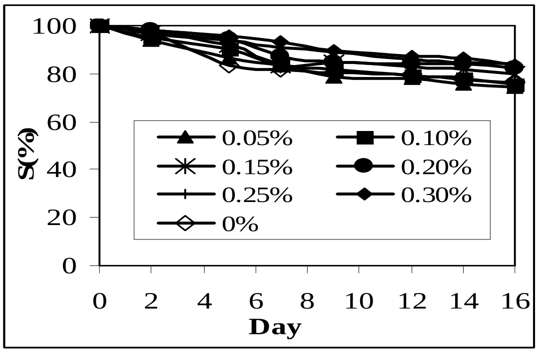

Figure 7. Effect of potassium sorbate concentration on the stability of 'submicron curcumin'

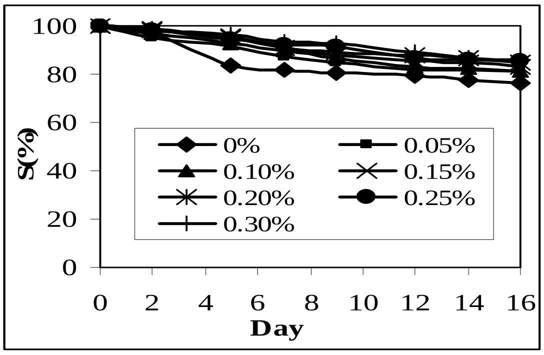

Figure 8. Effect of sodium sorbate concentration on the stability of 'submicron curcumin'

With this result in mind, we then investigated the stability of 'submicron curcumin' in the presence of some common ingredients that could be used or could be contaminated in food products. Parabens (methyl and propyl esters of *para*-hydroxybenzoic acid) are chemicals widely used as food-grade preservatives, and the U.S.A. Food and Drug Administration (FDA) allows certain preservatives to prevent microbial growth and preserve product integrity in cosmetics, medicines and food. In this research, it was found that the stability of 'submicron curcumin' remained almost unchanged in the presence of up to 0.02% methyl paraben, with 77% curcurmin being obtained after 16 days. Increasing the concentration of methyl paraben to 0.05% resulted in a slight drop in stability, with 68% curcumin being observed after 16 days (Figure 5). However, experimental results showed that up to 0.05% of propyl paraben could be used as a preservative for the 'submicron curcumin' suspension (Figure 6). This indicated that methyl paraben and propyl paraben could be used, if desired, in the product containing 'submicron curcumin'.

Potassium sorbate or sodium sorbate is an effective inhibitor of the growth of most moulds, yeasts and many bacteria. It is normally employed in meat processing or other food products to prevent microbiological growth and extend the normal life of food products. We therefore decided to investigate the stability of the 'submicron curcumin' suspension in the presence of potassium sorbate and sodium sorbate, respectively. Interestingly, it was found that the stability could be slightly improved under this condition. In the case of potassium sorbate, 84% curcumin was observed after 16 days at the potassium sorbate concentration of 0.3% (Figure 7). Using sodium sorbate at concentration of 0.3%, the stability of the 'submicron curcumin' suspension could be improved to 86% (Figure 8). This also indicated that potassium sorbate and sodium sorbate could be present in the product containing 'submicron curcumin' in aqueous media.

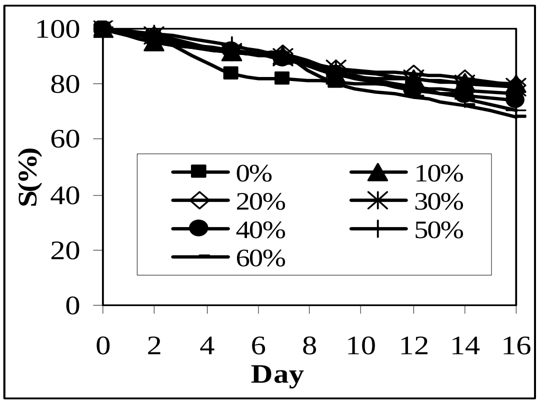

Figure 9. Effect of saccharose concentration on the stability of 'submicron curcumin'

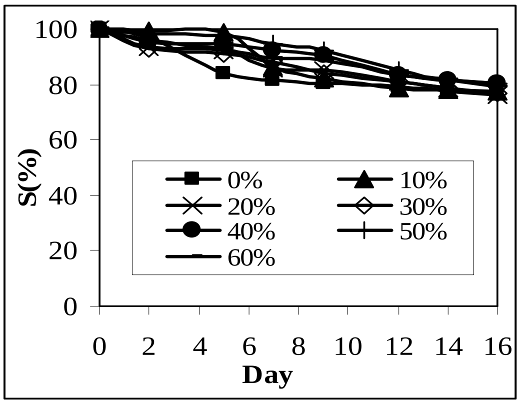

Figure 10. Effect of honey concentration on the stability of 'submicron curcumin'

One of popular ingredients that is widely used in several food products is saccharose sugar. The presence of sugar could affect the stability of the 'submicron curcumin' suspension. It was therefore decided to investigate its stability under this condition. It was observed that the stability of 'submicron curcumin' remained almost unchanged in the presence of up to 30% saccharose. Increasing the concentration of saccharose in the 'submicron curcumin' suspension to 40%, 50%, and 60% decreased its stability to 74%, 70%, and 68%, respectively (Figure 9). *Indeed, the mixture of curcumin and honey has been used in several traditional medicinal products*. Interestingly, in this research, it was found that the presence of honey did not affect the stability of the 'submicron curcumin' suspension at honey concentration of up to 60%, with 80% stability being observed after 16 days (Figure 10).

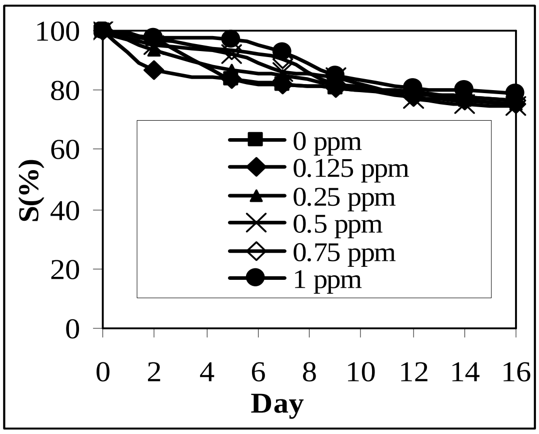

Figure 11. Effect of Cu^{2+} concentration on the stability of 'submicron curcumin'

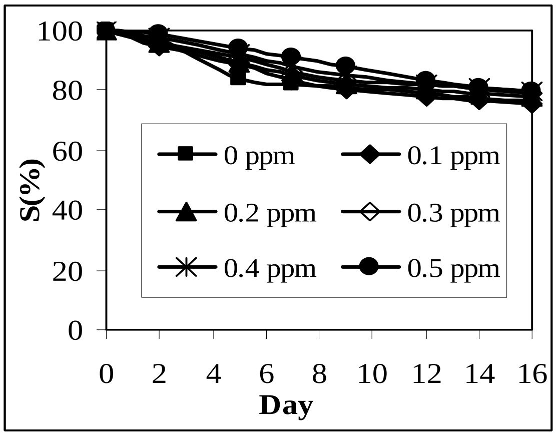

Figure 12. Effect of Fe^{2+} concentration on the stability of 'submicron curcumin'

The heavy metal cations in the water used to make the 'submicron curcumin' could also be a factor that should be taken into accounts. We therefore investigated the effect of two representative cations normally present in drinking water, including Cu^{2+} and Fe^{2+}. Based on the amounts of these cations detected in drinking water, concentration ranges of 0 – 1 ppm and 0 – 0.5 ppm were used for the case of Cu^{2+} and Fe^{2+}, respectively. Within experimental errors, it was found that the stability of the 'submicron curcumin' suspension remained almost unchanged after 16 days, with 80% stability being observed under these conditions (Figure 11 and 12). However, further research is needed to investigate the effect of other heavy metal cations present in the water on the stability of the 'submicron curcumin' suspension in aqueous media.

4. CONCLUSIONS

In summary, 'submicron curcumin' suspension in aqueous media was prepared from *Curcuma longa* L. without the presence of any additive. The average diameter of the 'submicron curcumin' particles was 546 nm (DLS). It was found that the 'submicron curcumin' suspension could be used in conjunction with several common food ingredients with its stability remaining unaffected. As the system only contained 'submicron curcumin' and water, it would be highly promising to applications in functional food, cosmetic, and pharmaceutical industries. Current research in our laboratory has been directed to the preparation and applications of several kinds of submicron and nanoparticle systems from bioactive natural products.

NGHIÊN CỨU ĐỘ BỀN CỦA 'SUBMICRON CURCUMIN' TỪ CỦ NGHỆ VÀNG *CURCUMA LONGA* L

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TÓM TẮT*: Curcumin tách từ củ nghệ vàng Curcuma Longa L. đã và đang được nghiên cứu ứng dụng trong nhiều lĩnh vực khác nhau như thực phẩm, mỹ phẩm và dược phẩm. Tuy nhiên, do curcumin rất ít tan trong nước nên hiệu quả sử dụng của curcumin trong những lĩnh vực này thực tế vẫn chưa cao như tiềm năng vốn có của nó. Trong nghiên cứu này, huyền phù của curcumin dạng kích thước submicron đã được điều chế trong dung môi là nước mà không cần phải thêm bất cứ phụ gia nào. Kích thước trung bình của các hạt phân tán curcumin vào khoảng 546 nm (xác định bằng phương pháp DLS). Kết quả nghiên cứu cho thấy có thể sử dụng huyền phù curcumin dạng submicron với một số phụ* *gia hoặc hóa chất thường gặp trong các sản phẩm thực phẩm mà độ bền của curcumin dạng submicron không bị ảnh hưởng. Hệ huyền phù chỉ chứa curcumin và nước, nên hứa hẹn có nhiều ứng dụng trong các lĩnh vực thực phẩm chức năng, mỹ phẩm và dược phẩm.*

Từ khóa: *Curcumin, củ nghệ vàng Curcuma Longa L.*

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