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PRELIMINARY STUDY ON DISPERSION OF α-MANGOSTIN IN PNIPAM MICROGEL SYSTEM

(Kajian awal penyebaran α-mangostin dalam sistem mikrogel PNIPAM)

Madihah Ahmad, Bohari M. Yamin* and Azwan Mat Lazim

School of Chemical Sciences and Food Technology,
Faculty of Science and Technology,
Universiti Kebangsaan Malaysia, 43600 UKM Bangi, Selangor, Malaysia

**Corresponding author: bohari@ukm.my*

Abstract

α-mangostin is a natural product containing xanthone core structure isolated from mangosteen rind. It is known to possess many biological properties including antibacterial, antiviral, anti-inflammatory and antioxidant activities. Some food products such as XanGo Mangosteen Juice, Vemma Mangosteen Juice Nutrition Supplement and Nanoxan Gold containing α-mangostin are readily available in the market. Spectroscopic study showed that α-mangostin exhibits a maximum fluorescence peak at approximately 500 nm which can be utilized for internal irradiation and photoimaging purposes. It may also have an effect on a formulated products containing α-mangostin. However, the emission peak for α-mangostin was not observed in the PNIPAM microgel solution. In the present study, α-mangostin was added into PNIPAM microgel solution. The dispersion of α-mangostin was studied by using Dynamic Light Scattering (DLS) and Transmission Electron Microscope (TEM).

Keywords: α-mangostin, PNIPAM microgel, Dynamic Light Scattering and Transmission Electron Microscope

Abstrak

α-Mangostin adalah sebatian semulajadi daripada kulit manggis yang mempunyai rangka xanthone dalam strukturnya. Sebatian ini memang telah diketahui mempunyai pelbagai sifat biologi seperti anti bakteria, anti virus, anti radang dan juga anti oksida. Beberapa produk yang mengandungi sebatian ini sebagai bahan utama seperti XanGo Mangosteen Juice, Vemma Mangosteen Juice Nutrition Supplement and Nanoxan Gold telah pun berada di pasaran. Kajian spektroskopi menunjukkan α-mangostin mempunyai puncak pancaran pendarflour pada kira-kira 500 nm. Kehadiran sifat ini boleh dimanfaatkan untuk aplikasi sinaran dalaman dan fotoimejan. Ia juga mungkin memberi kesan terhadap formulasi produk-produk yang mengandungi sebatian ini. Walau bagaimanapun, puncak pancaran bagi α-mangostin tidak lagi kelihatan di dalam larutan mikrogel PNIPAM. Dalam kajian ini, α-mangostin telah dicampurkan ke dalam larutan mikrogel PNIPAM. Penyebaran sebatian ini dalam sistem mikrogel telah dikaji menggunakan spektroskopi Serakan Cahaya Dinamik (DLS) dan Mikroskop Transmisi Elektron (TEM).

Kata kunci: : α-mangostin, Mikrogel PNIPAM, Serakan Cahaya Dinamik dan Mikroskop Transmisi Elektron

Introduction

Known as the "Queen of Tropical Fruits", the mangosteen is one of the most popular tropical fruits which easily can be found in South East Asia countries such as Malaysia, Thailand and Indonesia. The white flesh tastes very sweet and juicy, with a lemon tang. Since the discovery of α-mangostin in 1855 by W.Schmid [1] from the pericarp of *Garcinia mangostana* linn., it has been subjected to many studies not only on isolation and structural elucidation but also its biological and medical properties.

α-mangostin, a major component isolated from the pericarp of mangosteen fruit exhibits a wide spectrums of biological activities such as antioxidant [2], antimicrobial [3], anti-inflammatory [4], antifungal [5], and antibacterial [6]. To date, it has being used as a supplement in food products and natural dyes in fabric industries [7]. In fact, a juice containing α-mangostin as food supplement products have been patented [8] and now available in the market. Surprisingly, the general method involving the normal laboratory extraction and separation of the α-mangostin has also been patented [9]. Another property of α-mangostin is that it exhibits fluorescence spectrum that is potential in radiation therapy as well as photo imaging.

A major interest in microgels lies in their potential as novel drug delivery systems, incorporating drug molecules into the polymer network then releasing them at a targeted site within the body. The drug maybe encapsulated in a polymeric capsule or into a matrix tablet form. Another approach is making used of microgel systems in which the active ingredients bind to these micro-sized polymers. For example, PNIPAM (Poly-*N*-isopropylacrylamide) is one of the polymers which have been studied as a drug carrier [10]. This paper, explores preliminary study on α-mangostin dispersions in PNIPAM microgel systems.

Materials and Methods

Material and instrumentation

All chemicals and solvents used were of reagent grade and used without further purification. Infra-red spectra were recorded on a Perkin Elmer GX Spectrometer by using potassium bromide pellet. Ultra violet spectra were determined on a Shimadzu UV-VIS Spectrophotometer (UV 2400PC series). A Perkin Elmer LS-55 flouresence spectrophotometer was used for the excitation and fluoresence spectra. ^{1}H and ^{13}C NMR spectra were measured on Jeol JNM-ECP 400 NMR Spectrometer. Microgel particle sizes and polydispersities were determined by dynamic light scattering (DLS) using a Zetasizer Nano-S (Malvern,PA). The size and morphology of the sample was investigated by using Transmission Electron Microscope (TEM) Philips CM12 model.

Extraction of α-mangostin

Sampel of dried mangosteen rind was collected from Kuala Berang, Terengganu. Extraction of α-mangostin was carried out by following the normal procedure of isolating natural products as previously reported [11] . The grinded mangosteen rind was extracted with methanol for three weeks and then separated by column chromatography eluted with the mixture of dichloromethane-hexane (6:4) giving a fine yellow powder.

Synthesis of PNIPAM microgel

The PNIPAM microgels were synthesized by a surfactant free polymerization technique as previously reported [12].

Addition of α-mangostin on the PNIPAM microgel

About 0.08 g α-mangostin was dissolved in 10 ml ethanol and diluted to the concentration 5×10^{-5} M. Based on the volume of stock α-mangostin and PNIPAM microgel, a series of solution with different ratio, 1:1, 1:2, 1:3 and 1:4 ratio was prepared by mixing stock α-mangostin and stock PNIPAM. For 1:1 ratio, 3ml of the PNIPAM was mixed with 3 ml (5×10^{-5}M) of α-mangostin. The solution was stirred with magnetic stirer for 10 minutes. For the other 3 solutions of different ratios , adequate amount of α-mangostin solution was added to 3 ml of PNIPAM solution in a manner that the total volume of the solutions are sufficient for DLS measurement.

Results and Discussion

α-mangostin (Figure 1) was obtained as a yellow crystalline solid with melting point 175-178°C. The infra red spectrum showed the presence of stretching frequency of OH and C=O groups at 3421 and 1639 cm^{-1} respectively.

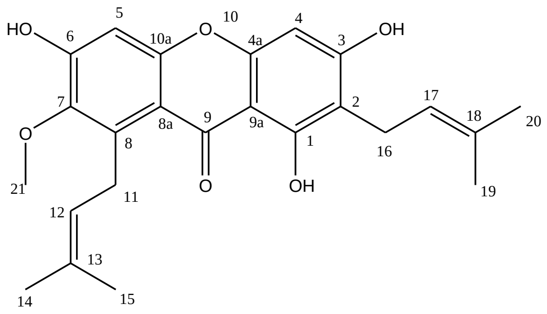

Figure 1. Structure of α-mangostin

The NMR results for the aromatic, olefinic and methoxy proton of the xanthone compound are shown in Table 1. As expected, all the ^{13}C chemical shifts of the xanthone were observed.

Table 1. ^1H NMR and ^{13}C chemical shifts of α-mangostin

C-position	^{13}C NMR	^1H NMR	C-position	^{13}C NMR	^1H NMR
1	160.1	13.69	10a	157.2	
2	109.9		11	18.3	3.98 (2H)
3	162.6	-	12	122.6	5.13 (1H)
4	92.5	6.33(1H, s)	13	130.9	
4a	154.5		14	26.0	1.59(3H,s)
5	102.0	6.77 (1H,s)	15	17.9	1.59(3H,s)
6	157.2	-	16	25.7	3.98 (2H)
7	143.6		17	123.9	5.13 (1H)
8	136.7		18	130.8	
8a	110.2		19	25.7	1.69(3H,s)
9	181.6		20	17.9	1.69(3H,s)

The UV-Vis spectra of α-mangostin (Figure 2) showed maximum absorption peak at 243, 317 and 352 nm are in agreement with the reported values [13].

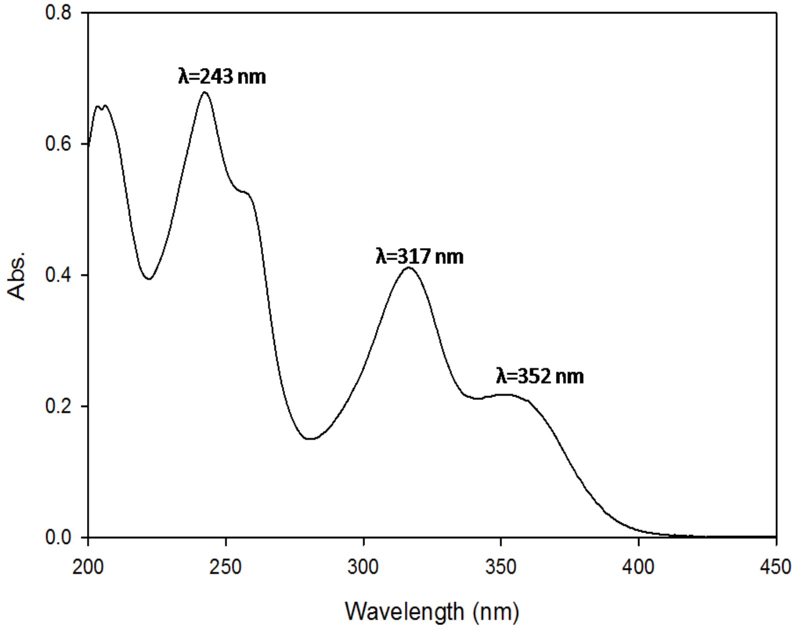

Figure 2. UV-Vis spectrum of α-mangostin in ethanol

Aqueous PNIPAM solution showed no UV-visible absorption peak above 210 nm. No significant changes for α-mangostin spectrum in the microgel system were observed except the peak at λ= 243 nm became less symmetrical due to overlapping of absorbance with the PNIPAM solution (Figure 3). The peaks at 352 nm also broaden. This may be due to an interactions or solvation effect between α-mangostin and microgel system. Such an observation has not yet been reported so far. Moreover, the fluorescence peak (λ=500 nm) was no longer observed in the mixture which also indicate the presence of an interaction between the α-mangostin and microgel structure.

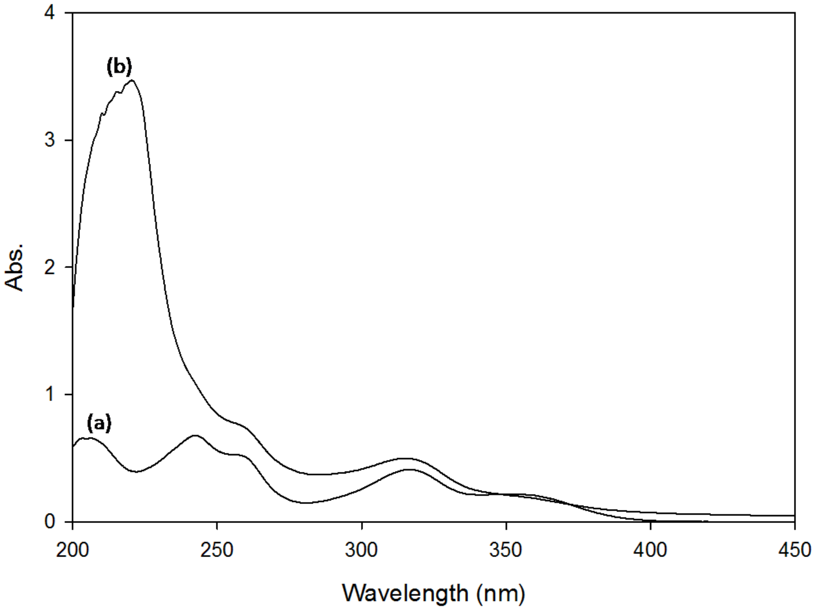

Figure 3. UV-Vis spectra of α-mangostin in ethanol (a) and in aqueous PNIPAM solution (b)

Dynamic Light Scattering (DLS) measurement of PNIPAM particles gave the average size of 1133 nm. The size became larger when the concentration of mangostin was increased (Table 2). This is consistent with the literature reports on the interaction of some neutral and charged additives with microgels [14, 15]. The increase in size may indicate the penetration of α-mangostin into the polymer network and cause an electrostatic repulsion which resulted the swelling of the PNIPAM microgel particles. It is also indicated a partially negative nature of the α-mangostin probably due to the carbonyl and hydroxyl groups. This also suggested that α-mangostin could have penetrated into the microgel core colloid. The mutual repulsion of respective charges consequently caused the size to increase. The swelling behavior observed is similar as reported on the addition of anionic sodium dodecyl sulfate (SDS) into ionic poly(*N*-vinylcaprolactam) (PNVC) microgels [16].

Table 2. Hydrodynamic diameter of PNIPAM particles in the presence of α-mangostin measured by DLS

Ratio PNIPAM : Mangostin	Dispersion size (nm)
PNIPAM	1133
1:1	1153
1:2	1557
1:3	1556
1:4	1641

The transmission electron microscope (TEM) image of the PNIPAM and α-mangostin solution showed a well dispersion of α-mangostin in microgel system (Figure 5). The drying of the sample for TEM experiment gave the nano sized of particles with the average diameter approximately 4 nm.

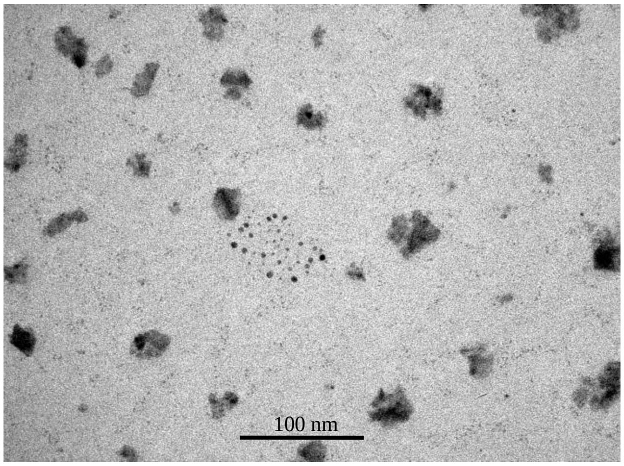

Figure 5. TEM images of PNIPAM with α-mangostin in 1:1 ratio

However, at high concentrations, the α-mangostin particles tend to form aggregation or cluster (Figure 6). The crystal like-particles also indicate the role of microgel as a medium for crystallization and under controlled conditions can be a method leading to the formation of nanocrystals.

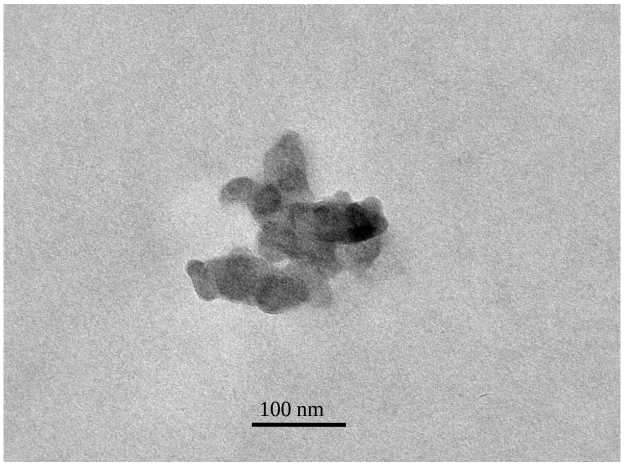

Figure 6**.** TEM images of PNIPAM with α-mangostin in 1:4 ratios

Conclusion

Ethanoic solution of α-mangostin showed a fluorescence peak at 500 nm. However, no fluorescence peak was observed in PNIPAM microgel solution. The presence of α-mangostin in PNIPAM microgel solution resulted in the increase of the colloidal size indicating a possible inclusion of the α-mangostin into the partially negative polymer shell and caused it to swell. TEM micrograph showed a well dispersed α-mangostin in the micogel at low concentrations but tend to aggregate or cluster formation at high concentrations. A somewhat defined shape of the xanthone aggregate indicates the role of the microgel as medium for crystallization.

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