

The Effect Of Temperature On The Dispersion of α -Mangostin In Pnipam Microgel System

Madihah Ahmad, Bohari M. Yamin² and Azwan Mat Lazim

*School of Chemical Sciences and Food Technology, Faculty of
Science and Technology, Universiti Kebangsaan Malaysia*

²Email: bohari@ukm.my

ABSTRACT

*α -Mangostin was extracted from the pericarp of the Malaysian local *Garcinia mangostana* linn., The structure was characterised by Infrared red, UV-Visible and Nuclear Magnetic Resonance spectroscopic data. The fluorescence peak at 500nm in ethanol was not observed in PNIPAM microgel solution. The increase of colloidal size of the gel in the presence of α -mangostin was studied by Dynamic Light Scattering and Transmission Electron Microscope. The size of the particle also increases with increasing temperature up to 45°C after which it began to shrink. The TEM micrograph at 45°C showed a uniformly structured pattern of the gel occurs in the range of the lowest solution critical temperature.*

Keywords: α -mangostin, PNIPAM microgel, Dynamic Light Scattering (DLS) and Transmission Electron Microscope (TEM). Lowest solution critical temperature (LSCT).

Introduction

α -Mangostin from the pericarp of *Garcinia mangostana* linn., has been subjected to many studies since its discovery in 1855 [1] not only on the isolation and structural elucidation but also its biological and medical properties. It was found to exhibit a wide spectrum of biological activities such as antioxidant [2], antimicrobial [3], anti-inflammatory [4], antifungal [5] and antibacterial [6]. It is also being used as a supplement in food products and natural dyes in fabric industries [7]. In fact, a patented juice containing α -mangostin [8] are now available in the market. Surprisingly, even the general method involving the normal laboratory extraction and separation of the α -mangostin has also been patented [9]. The fluorescence spectrum exhibited by α -mangostin is certainly has 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 may be encapsulated in a polymeric

capsule or into a matrix tablet form. Another approach is making use 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, is a continuation of our preliminary study on α -mangostin dispersions in PNIPAM microgel systems.

Experimental

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 fluorescence spectrophotometer was used for the excitation and fluorescence spectra. ^1H 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.

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Extraction of α -mangostin

Sampel of dried mangosteen rind were 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. Four solutions containing different ratio of PNIPAM and α -mangostin were prepared. 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 stirrer for 10 minutes. Three solutions of different ratios, were

prepared by adding the desired amount of α -mangostin solution into PNIPAM solution by keeping the total volume of 3 mL.

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.

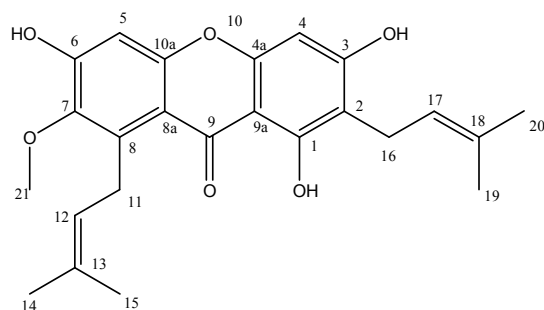


Figure 1: Structure of α -mangostin

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) shows maximum absorption peak at wavelength of 243, 317 and 352 nm are in agreement with the reported values [12].

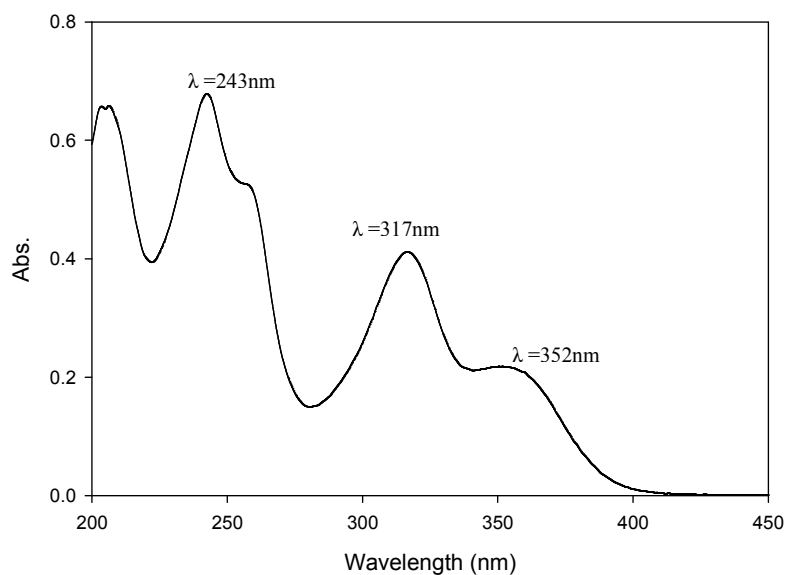


Figure 2: UV-Vis spectrum of α -mangostin in ethanol

Aqueous PNIPAM solution showed no UV-visible absorption peak at wavelengths above 210 nm. No significant changes for α -mangostin spectrum in the microgel system were observed except the peak at wavelength of 243 nm became less symmetrical due to overlapping of absorbance with the PNIPAM solution (Figure 3). The peaks at wavelength of 352 nm also widen and became less obvious. Therefore some kind of interaction must have taken place in the microgel systems. Moreover, the fluorescence peak ($\lambda=500\text{ nm}$) was no longer

observed in the mixture which also indicate the presence of an interaction between the α -mangostin and microgel structure.

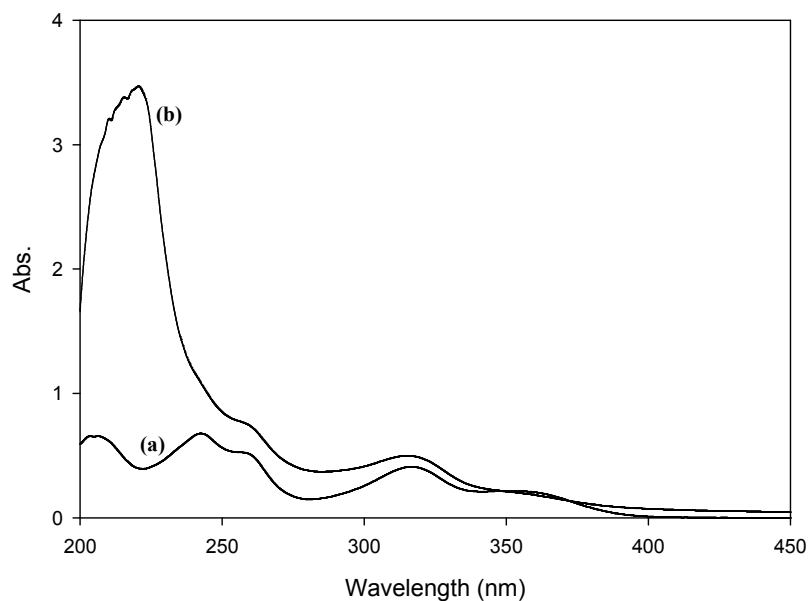


Figure 3: UV-Vis spectra of α -mangostin in ethanol (a) and in aqueous PNIPAM solution (b)

Dynamic Light Scattering (DLS) measurement of PNIPAM solutions gave the average size of 1133 nm. The size became larger when the concentration of mangostin was increased (Table 2). This is in consistent with the literature reports on the interaction of charged additives with PNIPAM microgels [14,15]. The increase of α -mangostin concentration

may contribute to the hydrophobic interactions of sulfate charged groups of PNIPAM that caused swelling of the microgel through electrostatic repulsion. It is also indicate 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.

Table 2: Dispersion size of PNIPAM in the presence of α -mangostin measured by DLS

Ratio PNIPAM : Mangostin	Dispersion size (nm)
PNIPAM	1133
1:1	1153
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 (Figure 5). The average diameter is approximately 4 nm.

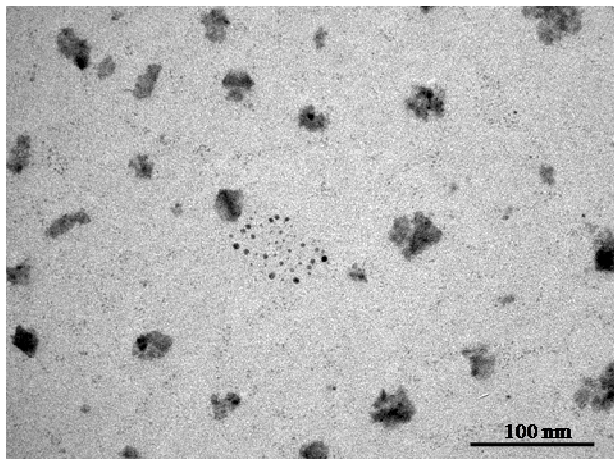


Figure 5: TEM images of PNIPAM with α -mangostin in 1:1 ratio

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.

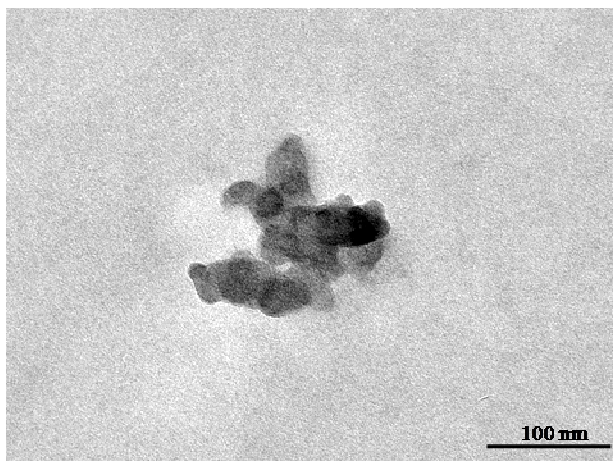


Figure 6: TEM images of PNIPAM with α -mangostin in 1:4 ratio

The effect of temperature

PNIPAM is a temperature responsive microgel. Since the 1:1 mixture of α -mangostin and PNIPAM showed good dispersion therefore this ratio was chosen for the temperature effect study. At room temperature DLS measurements gave particle size about 153 nm. The size increased to 1235 at 45°C (Table 3). It is known that aggregation of polymer occurs up to a critical value. For the PNIPAM , the lowest critical temperature (LCST) is reported between 30 and 45° C [16]. The hydrogen bonds formation with the water and intramolecular hydrophobic force also play important role. However, when the temperature reached around 55°C, the particle size started to decrease. At temperatures above the LCST the microgel particle will shrink.

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PNIPAM is a charged particle and at high temperature the particle diameter will decrease and consequently increase the surface charge density [12]. The solvent molecules will escape through the polymer and causes it more hydrophobic. As a result, the α -mangostin will be trapped in the colloidal structure.

Table 3: DLS size distribution of PNIPAM in the presence of α -mangostin (1:1) at different temperatures

Temperature (°C)	Disribution size
25	1153
30	1180
35	1206
40	1221
45	1200
50	800
55	263

The changes in particle size were also recorded by TEM micrographs. Figure 7 shows the distribution of particles at room temperature before heating.

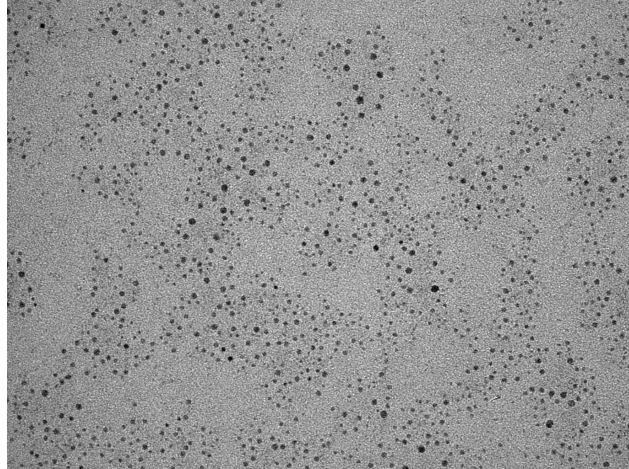


Figure 7: Distribution of α -mangostin/PNIPAM at 25°C before heating at 11500 magnification

As the temperature reached near the LCST, the microgel particles restructured to a nice regular pattern as shown in Fig.8. Due to the increment of the temperature, these particles deswelled and totally released all the solvent in their networks. As shown in Figure 9 the microgel particles shrunk and the result was in agreement with the DLS data (Table 3)

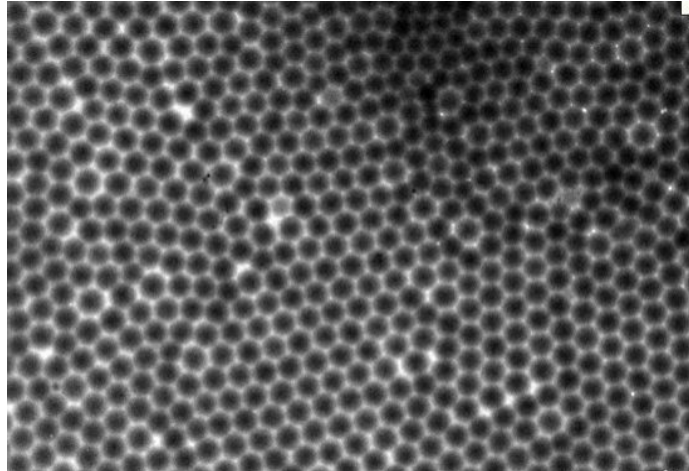


Figure 8: Distribution of α -mangostin/PNIPAM at 45°C close to LCST temperature. At 11500 magnification

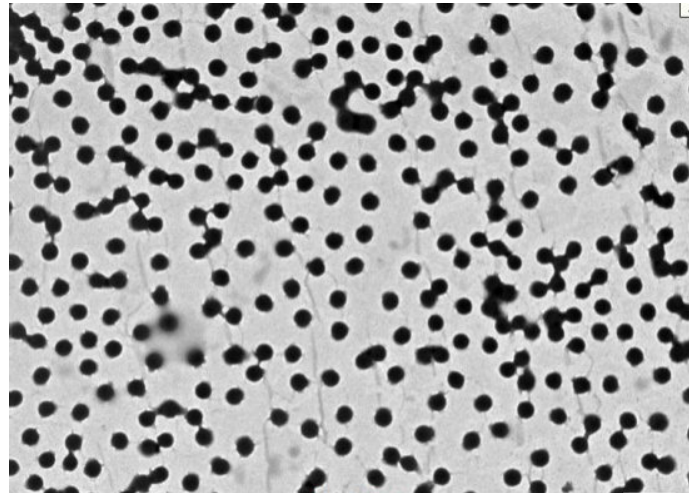


Figure 9: Distribution of α -mangostin/PNIPAM at 55°C above the LCST . At 11500 magnification

Conclusion

Fluorescence peak of α -mangostin at 500 nm was not observed in PNIPAM microgel solution. The increase of colloidal size indicating a possible inclusion of the α -mangostin into the partially negative polymer shell and caused it to swell. Both LDS measurement TEM micrograph showed a well dispersed α -mangostin and the low solution critical temperature is about 45°C and above this temperature the particle begin to shrink.

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References

- [1] Schmid, W. 1855. Ueber das mangostin. *Liebigs. Ann. Chem.* Vol. 93. pp. 83–89.

- [2] H.A. Jung, B. N. Su, W. J. Keller, R. G. Mehta & D. Kinghorn. 2006. Antioxidant Xanthones from pericarp of

Garcinia mangostana (Mangosteen). *J. Agric. Food.Chem.* Vol. 54. pp. 2077–2082.

[3] M. T. Chomnawang, S. Surassmo, V.S. Nukoolkarn & W. Gritsanapan. 2005. Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. *J. Ethnopharmacol.* Vol. 101. pp. 330-3.

[4] L.G.Chen, L.L.Yang & C.C. Wang. 2008. Antiinflammatory activity of mangostins from *Garcinia mangostana*. *Food Chem. Toxicol.* Vol.46. pp. 688-693.

[5] G.Gopalakrishnan, B. Banumathi & G. Suresh. 1997. Evaluation of the antifungal activity of natural xanthenes from *Garcinia mangostana* and their synthetic derivatives. *J. Nat. Prod.* Vol. 60. pp. 519-524.

[6] M. Iinuma, H. Tosa, T. Tanaka, F. Asai, Y. Kobayashi, R.Shimano & K. Miyauchi. 1996. Antibacterial activity of xanthenes from guttiferaceous plants against methicillin-resistant. *Staphylococcus aureus*. *J. Pharm. Pharmacol.* Vol. 48. pp. 861-865.

- [7] K. Siriwan. 2004. Using chitosan for improving the dyeability of cotton fabrics with mangosteen rind dye. *J.Sci.Res.Chula.Univ.*: Vol. 29(2).
- [8] K. B. Fugal, T. L. McCausland, X. Kou & W. J. Keller. 2006. Neutraceutical composition containing mangosteen pericarp extract. *United States Patent Application Publication*. Patent Pub. No.: US 2006/0088643.
- [9] R. Sobotta & H. P. Ignatow. 2006. Process for isolating of alpha-mangostin. *United States Patent Application Publication*. Paten Pub.No.:US 2006/0014967.
- [10] D. Schmaljohann. 2006. Thermo- and pH responsive polymers in drug delivery. *Advanced Drug Delivery Reviews*. Vol. 58. pp. 1655–1670.
- [11] J. Pedraza-Chaverri, L. M. Reyes-Fermin, E. G. Nolasco-Amaya, M. Orozco-Ibarra, O. N. Medina-Campos, O. Gonzalez-Cuahutencos, I. Rivero-Cruz & R. Mata. 2008. ROS Scavenging Capacity and Neuroprotective Effect of α -mangostin Against 3-nitropropionic acid in Cerebellar Granule Neurons. *Exp Toxicol Pathol*. Vol. 11.pp. 002.

- [12] R. J. Hall, V. T. Pinkrah, B. Z. Chowdhry & M. J. Snowden. 2004. *Colloids Surf., A*. 233.pp. 25–38.
- [13] L. Yu, M. Zhao, B. Yang, Q. Zhao & Y. Jiang. 2007. Phenolics from hull of *Garcinia mangostana* fruit and their antioxidant activities. *Food Chemistry*. Vol. 104. pp. 176–181.
- [14] K. C. Tam, S. Ragaram, R. H. Pelton. 1994. *Langmuir*. Vol. 10. pp. 418-422.
- [15] V. Neraspuri, J.L. Keddie, B. Vincent, & I. A. Bushnak. 2006. *Langmuir*. Vol. 22.pp. 5036-5041.
- [16]. S.H. Kim, I.J. Hwang, S.Y. Gwon, & Son, Y.A. 2010. The thermoresponsive behaviour of a poly(N-isopropylacrylamide) hydrogel with a D-p-A type pyran-based fluorescent dye. *Dyes and Pigments*. Vol. 87.pp. 84-88.