SUPPLEMENTAL INFORMATION

Controlled Curcumin Release via Conjugation into PBAE Nanogels Enhances Mitochondrial Protection against Oxidative Stress

Prachi Gupta[a],; Carolyn T. Jordan[a]; Mihail I. Mitov[b], D. Allan Butterfield[b,c]; J. Zach Hilt[a]; Thomas D. Dziubla[a]

[a]Chemical and Materials Engineering Department, F. Paul Anderson Tower, 512 Administration Drive, University of Kentucky, Lexington, KY 40506-0046 (USA)

[b]Redox Metabolism Shared Resource Facility, Markey Cancer Center, University of Kentucky, 1095 V.A. Drive, Health Sciences Research Building, Room 227, Lexington, KY-40536-0305 (USA)

[c]Department of Chemistry, Chemistry-Physics Building, University of Kentucky, Lexington, KY-40506 (USA)

Corresponding Author: Thomas Dziubla, dziubla@engr.uky.edu,

Phone: 859-257-4063

Fax: 859-323-1929

1 S. Curcumin nanogel size analysis in organic and aqueous medium

Curcumin acrylate (CA) was dissolved in anhydrous acetonitrile at a concentration of 5 mg/ml. 4,7,10-Trioxatridecane-1,13-diamine (TTD) in the ratio of 1:2 (CA v/s TTD) was added to the solution. The solution was mixed gently and allowed to react and precipitate into PBAE crosslinked nanogels for 24 hours. After the reaction, 100 µl of the nanogels suspension in acetonitrile was added to 4.9 ml fresh anhydrous acetonitrile followed by sonication. The suspension was then read using dynamic light scattering (DLS) zetasizer ZS90. Hydrodynamic diameter in tetrahydrofuran (THF) was measured after second wash of nanogels in THF. Post freeze-drying of nanogels after the THF washes, nanogels were suspended in PBS buffer (aided with 0.1% sodium dodecyl sulfate) to a concentration of 0.5 mg/ml. The system was sonicated using both probe and bath sonication and the diameter of the nanogels suspension was read at t = 0, 1, 5, 10 and 24 hour using DLS. The data recorded is shown in figure S1.

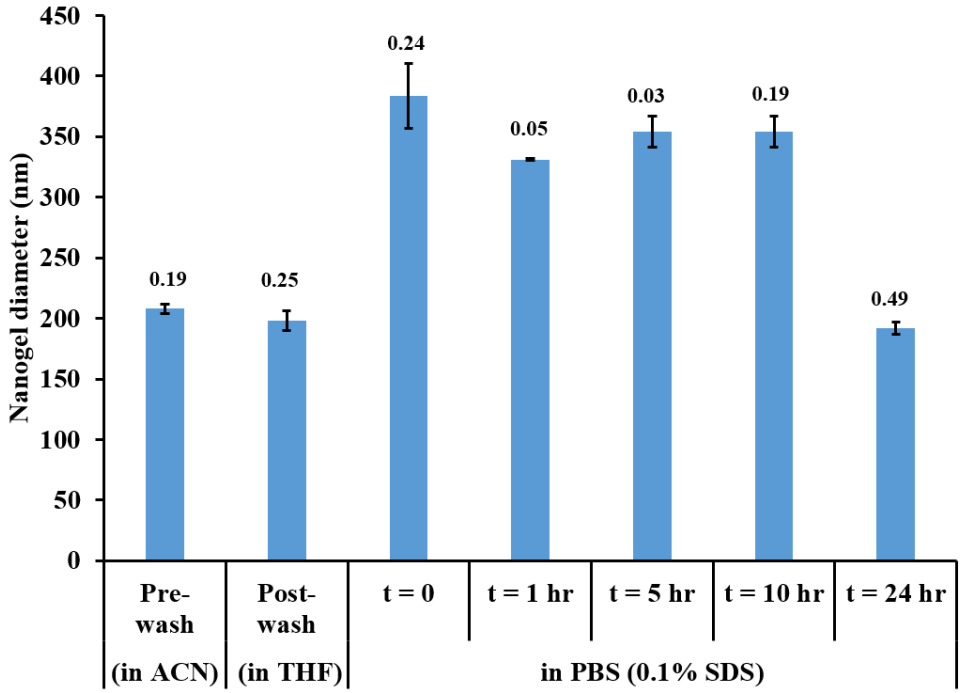

Figure1 S: Curcumin nanogel diameter pre-wash in acetonitrile, post-wash in THF and suspended in PBS-0.1% SDS buffer after freeze-drying. The values above each bar graph represents PDI for the corresponding sample group. N=3, Error bars: std. dev.

Increased size in PBS compared to in organic medium could be due to particle aggregation or swelling of gels. However, since the polydispersity index (PDI) of nanogels suspension in aqueous medium is low, effect of gel swelling could be one of the contributing factors.

1 S-a: Swelling study of curcumin nanogels in PBS

In order to capture the swelling profile of nanogels in PBS at early stage, curcumin nanogels suspension in acetonitrile obtained after 1st synthesis step was added to PBS buffer (aided with 0.1% SDS). To prepare the samples, 100 µl of the nanogels suspension in acetonitrile was added to 4.9 ml of PBS buffer, probe sonicated followed by time dependent size measurement. Figure 1S-a shows the hydrodynamic diameter with respect to time.

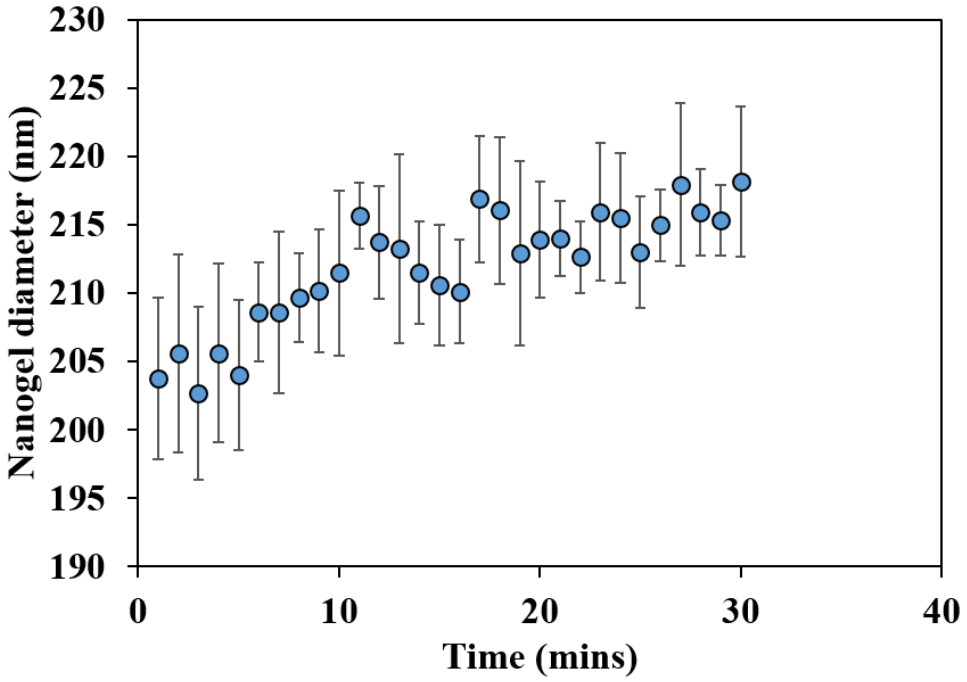

Figure 1 S-a: ***Curcumin nanogel (CNG) swelling in PBS.*** *Curcumin nanogel hydrodynamic diameter with respect to time. Suspension was prepared by adding 100 µl of nanogel suspension in acetonitrile obtained after 1st synthesis step to 4.9 ml of PBS buffer with 0.1% SDS followed by probe sonication. N=3, error bars: Std. err.*

2S: Curcumin stability test in PBS

A solution of curcumin in anhydrous DMSO at a concentration of 100 mg/ml was prepared. 10 µl of the solution was added to 990 µl of PBS buffer (aided with 0.1% SDS) resulting in 100 µg/ml of concentration. 30 µl of this solution was added to 970 µl of PBS buffer (0.1% SDS). The solution was then analyzed under UV-Vis to determine concentration with respect to time. The curcumin solution was read at 420 nm. Figure 2S shows the time dependent stability of curcumin in PBS in terms of concentration as µg/ml.

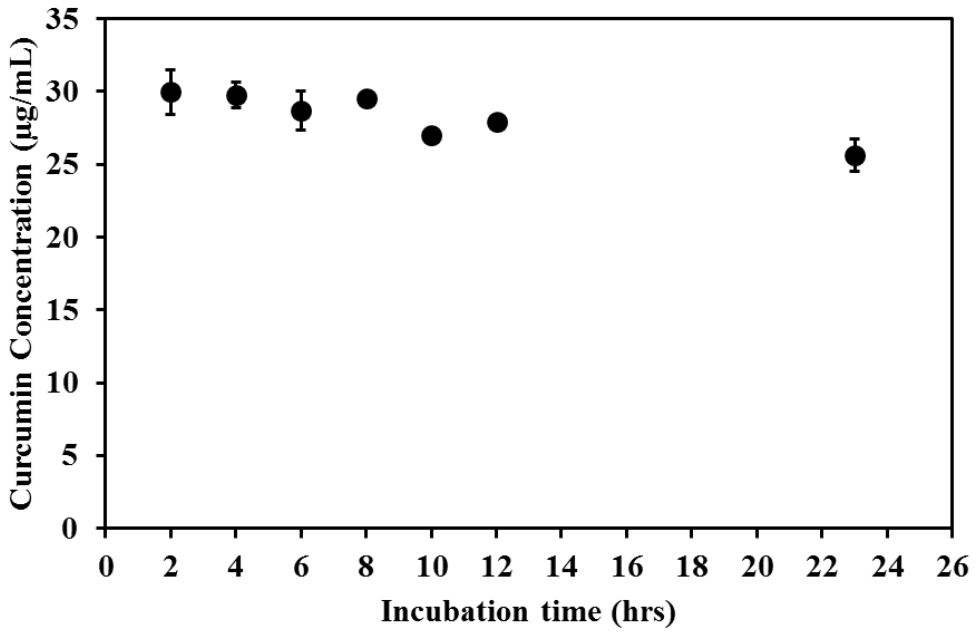

Figure 2 S: ***Curcumin stability test in PBS (0.1% SDS) at 37˚C for 24 hours.*** *A bulk solution of curcumin in DMSO was diluted to 30 µg/ml in PBS buffer. The buffer was aided with 0.1% SDS vol/vol in order to enhance the aqueous solubility. At set time points, the absorbance of the solution was read at 420 nm using Varian Cary 50 Bio UV-Vis spectrophotometer. N=3, Error bars: std. dev.*