Supporting Information

Microfluidic fabrication of cationic curcumin nanoparticles as an anti-cancer agent

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Materials and Methods:

Curcumin (95% pure) was obtained from Sabinsa Corporation (Piscataway, NJ) and used as received. Pluronic F-127 was purchased from BASF, Germany and didodecyl-dimethylammonium bromide (DDAB) was purchased from SIGMA-ALDRICH. Water with a resistivity greater than 18 MΩ.cm was acquired from a Millipure Milli-Q system and used for all experiments. Curcumin was dissolved in 0.1 M NaOH (500μM) along with DDAB and the non-ionic surfactant. A 0.1 M citric acid/tri-sodium citrate solution (pH 3.2) was used as the anti-solvent to precipitate the curcumin. A microfluidic continuous flow rotating tube processor (RTP) was used to prepare the curcumin nanopartciles; a set flow of liquids were introduced from a conical inlet at one end of the tube, with the discharged liquid collected by a peripheral ring at the other end. Integrated feed pumps were used to deliver the solution of the curcumin and acid solution, with flow rates at 0.3 ml/s and 0.6 ml/s respectively. During the injection process the mixed solution was homogenized at 1000 rpm, G force: 67.2 g. To evaluate the redispersibility and to study the physiochemical properties of the nanoparticles, the resulting nanoparticles were freeze-dried (Labconco freeze dryer model 75035, Labconco Corporation, Kansas City, MO).

A range of conditions were studied in generating the curcumin nanoparticles as listed in Table 1, with a concentration of the curcumin maintained throughout the experiments at 500μM.*

No	Curcumin solution* flow rate (mL/sec)	Surfactant		Antisolvent flow rate (mL/sec)	Rotation speed (RPM)	Mean Particle Size & Poly Dispersity Index(PDI)	Zeta Potential (mV) at pH 6.5
		Type	Con (%wt)				
1	0.3	-	-	0.6	100	250(15) 0.080	-3(6)
2	0.3	Pluronic F-127	0.5	0.6	1000	145(10) 0.092	-23(5)
3	0.3	DDAB	0.5	0.6	1000	80(20) 0.098	53(3)
4	0.3	DDAB-Pluronic F-127	0.5-0.5	0.6	1000	50(15) 0.034	48(5)

* All determinations were performed in triplicate and values are expressed as mean ± SD, $n = 3$.

DDAB plus pluronic F-127 was the surfactant of choice for fabricating the curcumin nanoparticles, being effective in forming small size particles.

Characterization for bulk measurement of the particles involved Dynamic Light Scattering (DLS) and Differential Scanning Calorimetry (DSC). The size and morphology were examined using Transmission Electron Microscopy (TEM) and Atomic Force Microscopy (AFM). For TEM analyses the samples were stained with 0.5% (w/v) phosphotungstic acid and fixed on copper grids before analysis. AFM measurements were made on an AFM D5000 instrument, with samples prepared by dispersing the curcumin nanoparticles with distilled water, followed by deposition of 10 μl of the colloidal suspension onto freshly cleaved mica plates, dried over 24 h at 25^{o}C.

Thermal properties of curcumin nanoparticles were investigated using DSC (model 823, Mettler-Toledo Instruments, Columbus, OH), with powder samples (ca 5 mg) placed in perforated aluminum sealed pans. The thermal analyses were performed with a temperature scanning range from 25 to 200 °C and a heating rate of 5°C/min. Nitrogen was used as the inert gas. The melting temperature of curcumin was recorded as the maximum peak temperature.

The dissolution of curcumin nanoparticles, micron size particles and physical mixtures was determined using a Hanson Model SR2, USA instrument. The dissolution media consisted of 500 ml of simulated gastric fluid without pepsin, at a pH 6.5. The paddles were rotated at 50(1) rpm and the temperature was maintained at 37.0(5)°C. The amount of each sample was equivalent to 10 mg of curcumin dispersed in the dissolution medium. A 5 ml aliquot was withdrawn at appropriate time intervals, filtered, diluted with dissolution medium and replaced with a 5 ml of freshly prepared dissolution medium after each sampling to maintain a constant volume. The amount of curcumin present was determined spectrophotometrically at 427 nm and 432 nm for simulated gastric fluid without pepsin for curcumin and nanocurcumin respectively. Curcumin concentration was calculated and expressed as a percentage of curcumin dissolved from the mean of six determinations.

Curcumin nanoparticles were stored at room temperature for two months. The physical stability of the material was then studied using DLS and DSC. There was not apparent change on the particle size and DSC thermographs Fig.S1.

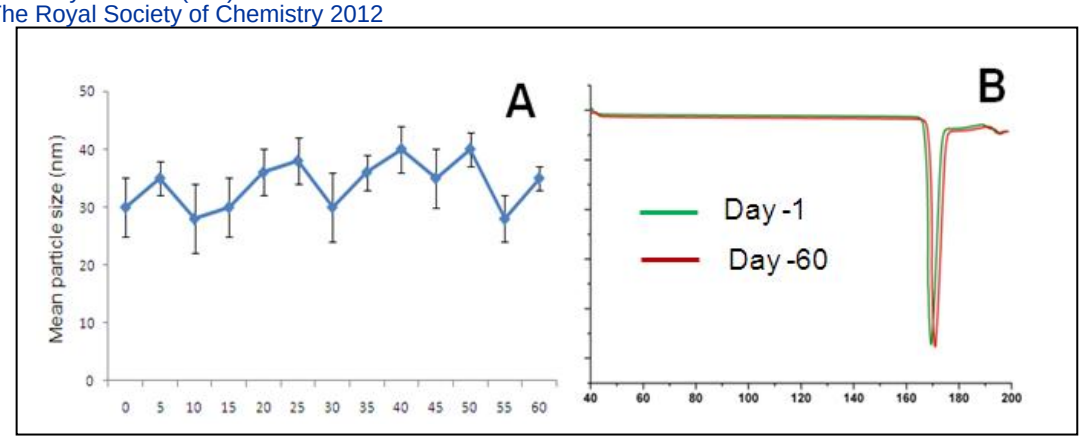

Fig. **S1** (A) DLS particle size measurements showing the physical stability of curcumin nanoparticles stored at room temperature, and (B) DSC thermographs of curcumin nanoparticles at zero day (Green) and after sixty days (Red).

Steady-state fluorescence measurements of curcumin were recorded using a Spex FluoroMax-3 spectrofluorimeter, with the concentration of the compound set at 10 μM. The emission spectra (Fig.2) were recorded from 450 to 700 nm with an excitation wavelength of 420 nm.

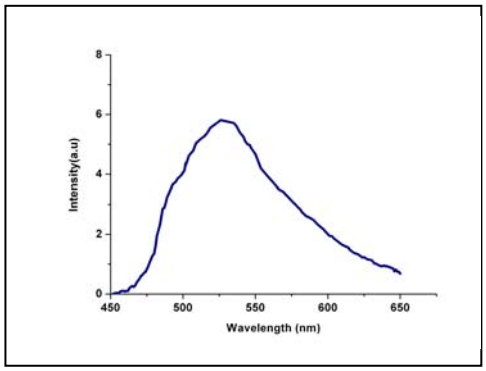

Fig. **2** Fluorescence spectrum of nanopartciles of curcumin stabilized with DDAB-Pluronic F 127 in aqueous solutions (Excitation wavelength at 420 nm).

Breast cancer cell lines MDA MB 438 and MCF-7 were obtained from American Type Culture Collection (HB-8065, Manassas, VA) and were cultured in DMEM/F12+GlutaMAX^{TM} medium (Invitrogen, Carlsbad, CA) containing 10% foetal bovine serum (Invitrogen, Carlsbad, CA), 100 units/mL penicillin (Invitrogen, Carlsbad, CA) and 100 μg/mL streptomycin (Invitrogen, Carlsbad, CA). Cells were maintained in incubators at 37°C under 95% relative humidity and 5% CO2. The anti-cancer activity of curcumin was examined using the (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) (MTS) assay. Briefly, MDA MB 438 and MCF-7 cells were

seeded in 96-well microtiter plates at a density of 5,000 cells/per well in a final volume of 100 µL medium. After 24 h, the cells were treated with a medium containing DMSO-dissolved or DDAB-pluronic F127-encapsulated curcumin at different concentrations. Other cells were untreated as negative control, or treated only with DMSO or DDAB plus pluronic F127 at the maximum concentration used to dissolve and encapsulate curcumin, respectively. After 72 h, the MTS solution (Cell Titer 96 Aqueous One Solution Reagent (Promega)) was added at 5 µl per well and incubated for 4 hours at 37°C. Absorbance at 490 nm was recorded with an Absorbance Microplate Reader (Molecular Devices, Sunnyvale, CA). Relative cell viability was expressed as A560–A670 normalised to that of the untreated wells. Data were presented as mean standard deviation with four-well repeats.