Enhanced fluorescence quenching of hemin detected by a novel polymer of curcumin†

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Electronic Supplementary Information (ESI)

Preparation of curcumin methacrylate:

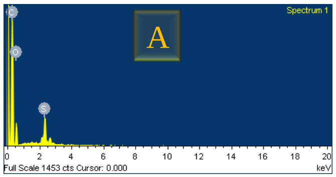
Methacrylic acid and benzoyl chloride are mixed in 1:2 molar ratios and the mixture is distilled at its boiling point in a round bottom flask in presence of small amount of hydroquinone. The distilled product is collected in a collector at ice cool temperature. This product is the required methacryloyl chloride and is redistilled for further purification.

To prepare curcumin methacrylate 1 g mol of curcumin is dissolved in ethyl methyl ketone in a two necked round bottom flask. 2 g mol of methacryloyl chloride along with 25 ml of ethyl methyl ketone are added drop wise into the flask with continuous stirring in an ice bath. 1.1 g mol of triethyl amine is then added to this reaction mixture to neutralize the acid produced in the reaction. After about 4 hours the mixture solution is filtered and the precipitate is washed with Milli pore water and further by 0.1 N NaOH solution. The organic layer produced after filtration is separated by a separating funnel. The dissolved product curcumin methacrylate is obtained by heating the viscous solution to about 100^{o}C. The product is washed with methanol and Milli pore water to remove impurities.

Results of EDX and GPC measurements:

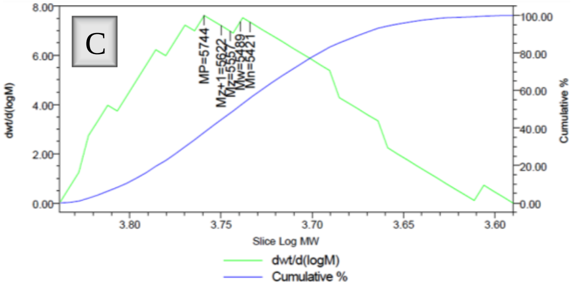
From EDX measurements with the help of Carl Zeiss Σigma VP using EHT 20kV, we determined the percentage composition of the polymer. It is observed that the results of repeating EDX record are very close to each other i.e. the element percentage are almost same. The EDX spectrum of the polymer is shown in supplementary figure S1 A and the percentage of elements are given in supplementary figure S1 B. We did not find unnecessary elements like N, Cl etc, which are generally detected under EDX measurements.

The molecular weight distribution is determined in THF by three column GPC system (WATERS 2414). The distribution curve obtained from GPC measurements and the molecular weights of the polymer are given in supplementary figure S1 C and D respectively. The molecular weight distribution is only two and both of the fragments possess a unit polydispersity index (PDI), i.e. only one monomer constitutes the whole polymeric structure.



B

Element	Weight%	Atomic%
C	76.06	81.77
O	21.23	17.14
S	2.81	1.09
Total	100	



D

	Dist Name	Mn	Mw	MP	Mz	Mz+1	Mv	Polydispersity	MW Marker 1	MW Marker 2
1		5421	5489	5744	5557	5622		1.012709		
2		2801	2802	2836	2802	2802		1.000148		

Supplementary Figure S1: A. SEM-EDX plot, B. % distribution of elements in sulfonated polymer of curcumin, C. GPC distribution plot for the polymer and D. GPC Results.

Estimation of sulfure: Estimation of sulfure involves the following steps

Firstly the known amount of the samples (0.01, 0.03 and 0.05 g of the samples is heated with fuming nitric acid. During this reaction H_2SO_4 is produced and its is make up to 50 ml. These solutions are further used for sulfure estimation.

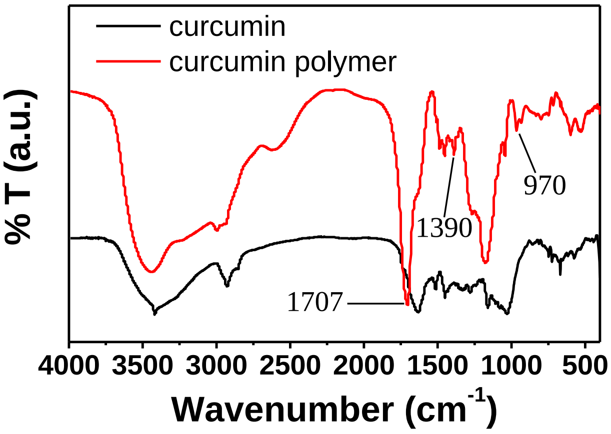
Secondly, a standard solution of Na_2SO_4 is prepared by dissolving 0.036 mg of it in 250 ml of distilled water. From this stock solution eight different solutions are prepared. A conditioning reagent is also prepared by mixing 75 g of NaCl in 30 mL of conc. HCl, 100 ml of 95% isopropyl alcohol in 300 ml of distilled water. To it 50 ml of glycerol is added and mixed thoroughly.

To each 50 ml of the Na_2SO_4 solutions 5 ml of the prepared conditioning solution is added and stirred well. During stirring, 0.2 g of $BaCl_2$ is added to each of the solutions and the reading of the UV-Vis spectrophotometer is collected at 420 nm. It produces a standard curve from which the concentration of sulfure present in the sample is calculated.

From this method the percentage of sulfure is found to be exactly 2.8 % as found in EDX measurements.

FT-IR spectroscopic studies:

FT-IR spectroscopic studies are done to investigate the change in functionality in curcumin after polymerization and the spectra are shown in supplementary figure S2. The spectra are recorded with the help of NICOLET 6700 FT-IR in transmittance mode over 32 scans.



Supplementary Figure S2: Comparison of FT-IR spectra of curcumin and the curcumin polymer.

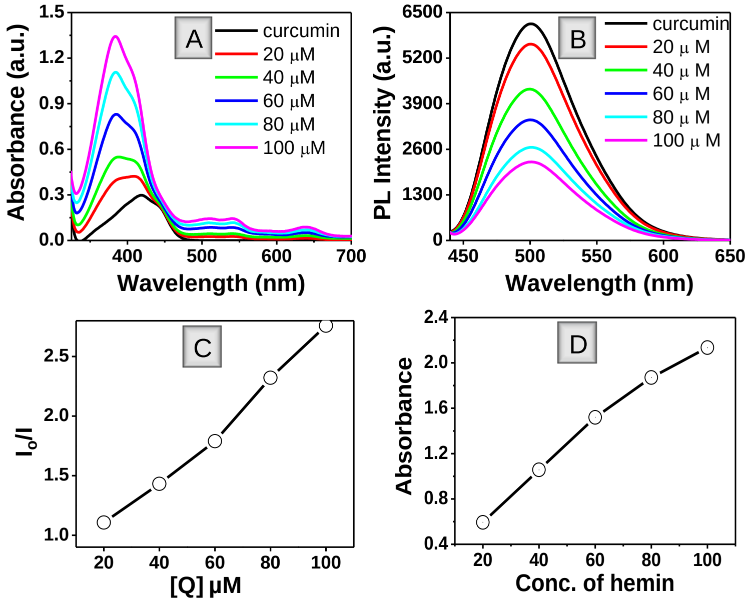
After polymerization some major change in FT-IR spectra is observed due to the appearance of new peaks for ester carbonyl as well as for the S=O bonds. There is no appreciable shift in peak positions for the rest of the spectra indicating no change in functionalities after polymerization. The O-H stretching vibration in both cases is observed at 3400 cm^{-1}. C-H stretching vibration for sp^2 and sp^3 hybridization are observed at around 3000 and 2928 cm^{-1}. Appearance of the new peak at around 1707 cm^{-1} in the polymer corresponds for symmetric stretching of C=O bond of the newly formed ester group.[1] After that two important peaks in both curcumin and the polymer are observed at around 1630 $cm^{-1}$and 1510 cm^{-1}, which are assigned as the stretching vibration of C=C group in extended conjugation and aromatic C-O stretching vibration respectively.[2] Aromatic C=C stretching in both curcumin and the curcumin polymer are present at the range of 1440-1480cm^{-1}.[3] Another new peak is observed in the curcumin polymer at around 1390 cm^{-1} and corresponds for asymmetric S=O stretching band which is not present in curcumin.[4] In plane C-H bending vibration is observed in both curcumin and in the curcumin polymer at around 1270 cm^{-1}.[5] This peak is more intense in the polymer as it has more number of C-H bonds after introducing aliphatic groups. In the polymer another new peak is present at 970 cm^{-1} which is due to ester C-O single bond stretching vibration.[1] Very small peaks at around 800 cm^{-1} is due to out of plane bending vibration of aromatic rings.

Quenching of fluorescence of curcumin in presence of hemin:

All the fluorescence measurements are carried out in Hitachi F-2500 spectrophotometer. The fluorescence quenching of curcumin is given in Figure S3 A. The quenching efficiency is comparatively low (63.05%) compared to the polymer as mentioned in the main article. The observed fluorescence quenching with increasing concentration of hemin is only due to collision between the fluorophore and the quencher which was also confirmed by the linear SV plot of curcumin.

UV-Vis spectra and PL spectra of curcumin in increasing conc. of hemin:

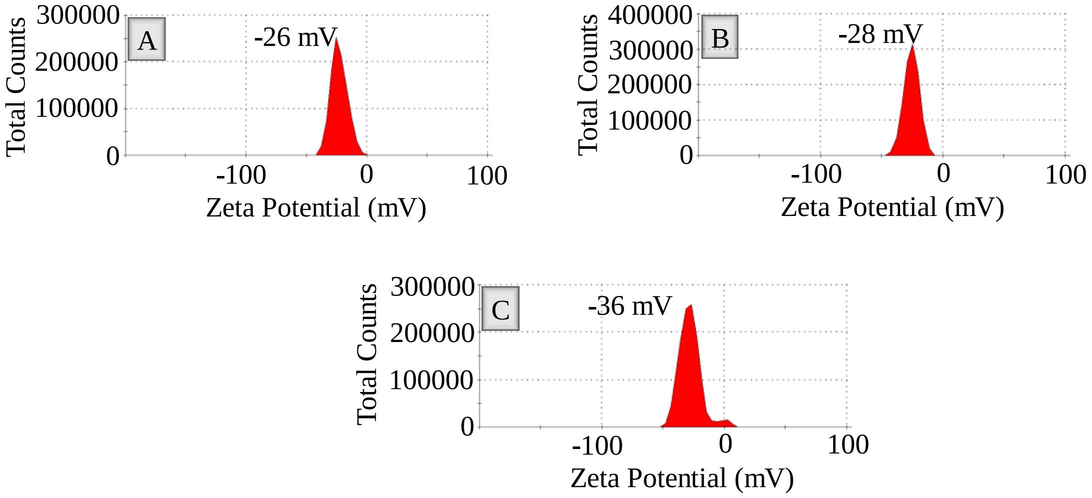
The UV-Vis spectra of curcumin possesses its characteristic peaks at 419 nm which is due to π-π* transition of the extended π-conjugation. A very weak electronic dipole forbidden n–π* transition of the carbonyl group of curcumin is located to the longer wavelength side.



Supplementary Figure S3: A. UV-Vis spectra and B. PL spectra of curcumin in increasing conc. of hemin, C. Linear SV-plot of curcumin, D. Calibration curve obtained from UV-Vis spectra of the polymer with hemin.

Zeta potential distribution:

The average zeta potential values of different components (curcumin, hemin, curcumin polymer etc) is determined with the help of Malvern Nano ZS90 and the plots are given in Supplementary Figure S3. By determining the change in average zeta potential values of curcumin and the polymer in presence of hemin we confirmed the possible charge transfer between the curcumin polymer and hemin. In the main manuscript we described how the zeta potential value of the curcumin polymer changes in presence of hemin. From Supplementary Figure S3 A and B it is evident that for curcumin there is no appreciable change in its average zeta potential value in presence of hemin as the difference between these values for curcumin (-26 mV) and hemin (-36 mV) are not enough to occur charge transfer between them.



Supplementary Figure S4: Zeta potential distribution curves for A. Curcumin alone, B. Curcumin with hemin and C. Hemin alone.

Interference of different chemicals:

We have investigated the interference of different chemicals like hemin, cysteine, aspartic acid, retinol, ammonia, chloroform, acetone, acetic acid and the some common ions like K^+, Na^+, Fe^{2+} etc as shown in Fig. S4. A bit decrease in quenching efficiency is observed in the presence of hemin, cysteine, aspartic acid, retinol, ammonia, acetone and acetic acid when the hemin conc. is 100 µM. However no such decrease in quenching efficiency is observed for the presence of common cations.

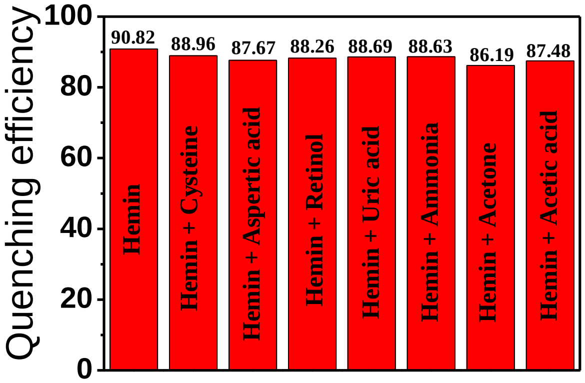


Fig S5: Interference of other chemicals in the quenching of the polymer by hemin.

Table S1: *t*-test results for three replicate measurements at 100 µM conc. of hemin

(A)

Data	Mean	Variance	N
Readings 1	143.50323	313654.12272	391
Readings 2	112.50323	313654.12272	391

At the 0.05 level,
The two means are NOT significantly different.

(B)

Data	Mean	Variance	N
Readings 2	112.50323	313654.12272	391
Readings 3	163.50323	313654.12272	391

At the 0.05 level,
The two means are NOT significantly different.

(C)

Data	Mean	Variance	N
Readings 1	143.50323	313654.12272	391
Readings 3	163.50323	313654.12272	391

At the 0.05 level,
The two means are NOT significantly different.

Table S2: Determination of standard error between the reference conc. of hemin to that of the mean of the taken con.

Reference conc. (µM)	**Mean of (µM)**	**Standard error**
40	20, 40, and 60	3.19
60	40, 60 and 80	2.38
80	60, 80 and 100	1.53