Electronic Supporting Information

Dendritic Star Polymer of Polyacrylamide Based on β-cyclodextrin Trimer: A Flocculant and Drug Vehicle

Kranthikumar Tungala[a], Pubali Adhikary[a], Venkanna Azmeera[a], Krishna Kumar[b] and S. Krishnamoorthi[a]*

[a]*Department of Chemistry, Centre of Advanced Studies, Institute of Science, Banaras Hindu University, Varanasi -221005, INDIA*

[b]*Department of Applied Science, Madan Mohan Malviya University of Technology, Gorakhpur-273010, INDIA*

*****Corresponding author: Email: dr.skmoorthi@gmail.com; Phone: +91 5426702484.

Synthesis of homopolymer of acrylamide (PAM)

Polyacrylamide was synthesized by solution polymerization technique, using $K_2S_2O_8$ as initiator. Acrylamide (2 g, 28.14 mmol, 300 equivalents) was dissolved in 10 mL of water and the mixture was stirred continuously for 15 minutes under nitrogen gas purging and heating up to 55 °C. $K_2S_2O_8$ (25 mg, 0.09 mmol, 1 equivalent) was dissolved in 2 mL water and added in to the reaction mixture. The reaction was carried out until the solution became viscous. The reaction mixture was cooled to room temperature and polymer was precipitated with acetone. The resulting polymer was dried under vacuum at 50 °C (Yield 1.82g, 91%).

Synthesis of star polymer of polyacrylamide based on β-CD (β-CD-PAM)

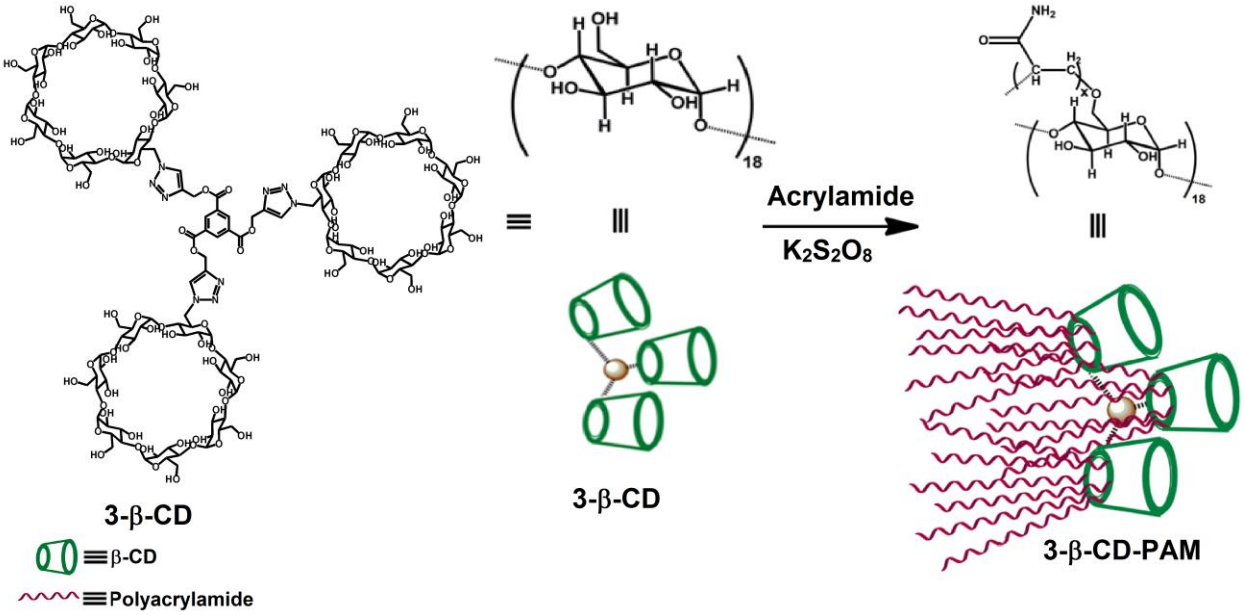
In a 100 mL two necked round bottom flask, β-cyclodextrin (227 mg, 0.2 mmol, 2 equivalents) was dissolved in 10 mL of water at 55 °C under continuous purging of nitrogen gas. After the

suspension became transparent, $K_2S_2O_8$ (190 mg, 0.7 mmol, 7 equivalent) dissolved in an adequate (2 mL) amount of water was injected to the reaction mixture in hot condition to initiate the free radical sites on the alcoholic groups of β-CD. After 5 minutes, acrylamide (4.97g, 70 mmol, 700 equivalents) dissolved in 5 mL of water was injected into the reaction mixture. Within few minutes reaction mixture became viscous and hydroquinone was added to quench the polymerization. The mixture was cooled to room temperature and precipitated in excess of acetone. The resulting polymer was washed with 1:1 solution of acetic acid and formaldehyde in order to remove any traces amount of homopolymer of acrylamide present in the mixture. The polymer, thus obtained, was dried under vacuum at 50 °C until constant weight (Yield 4.2g, 81%).

Synthesis of dendritic star polymer of polyacrylamide based on β-CD trimer (3-β-CD-PAM)

β-CD trimer, which was synthesized through click reaction, was utilized in synthesis of dendritic star polymer of acrylamide. For this, in a 50 mL two necked RB flask equipped with nitrogen gas purging, β-CD trimer (96 mg, 0.025 mmol, 1 equivalent) was dissolved in 5 mL of water. Then $K_2S_2O_8$ (61 mg, 0.225 mmol, 9 equivalents) dissolved in 2 mL of water was injected to the reaction mixture at 55 °C under continuous nitrogen gas purging. After 5 minutes, acrylamide (1.6 g, 22.5 mmol, 900 equivalents) dissolved in 3 mL of water was injected into the reaction mixture. As soon as reaction mixture became viscous, hydroquinone was added to terminate polymerization. The reaction mixture was cooled to room temperature. The synthesized polymer was precipitated in excess of acetone and the resulting polymer was washed with 1:1 solution of acetic acid and formaldehyde in order to remove any traces amount of homopolymer of

acrylamide present in the mixture. The star polymer, thus obtained, was dried in vacuum oven at 50 °C until a constant weight was obtained (Yield 1.4g, 83%).



Scheme S1. Schematic representation of synthesis of 3-β-CD-PAM.

^{1}H NMR and FTIR spectra of PAM, β-CD-PAM and 3-β-CD-PAM

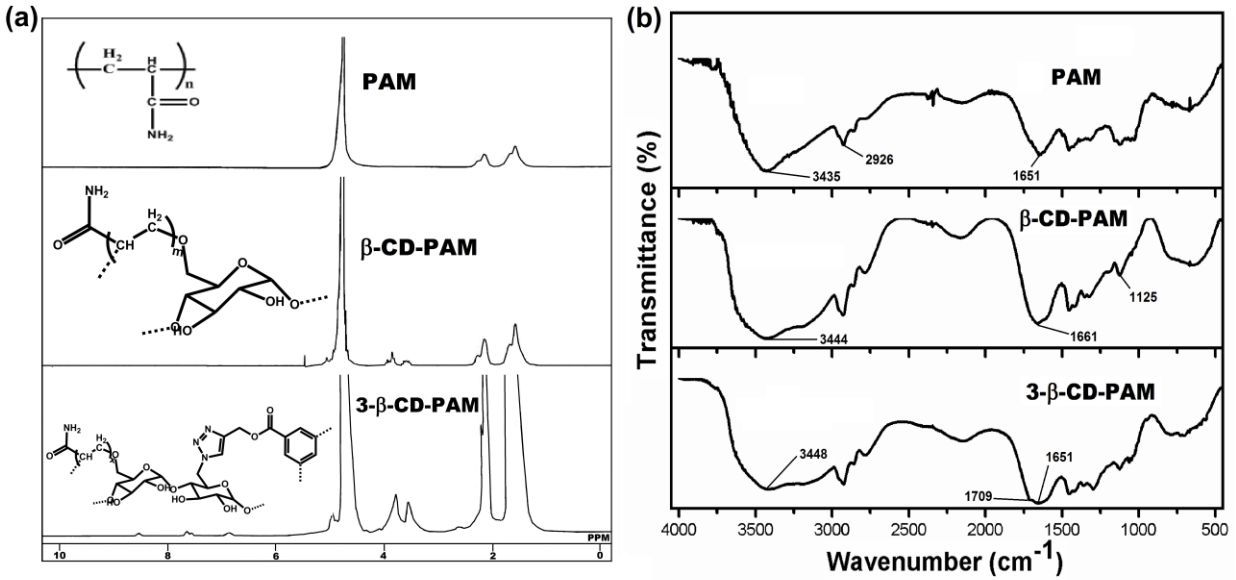


Fig. S1(a). ^{1}H NMR and **(b)** FT-IR spectra of PAM, β-CD-PAM and 3-β-CD-PAM.

The ^{1}H NMR of PAM revealed its characteristic peaks of $–CH_2$ protons at 1.52 ppm and –CH proton signals at 2.08 ppm. In FTIR, the characteristic signals of C=O stretching and N-H bending of polyacrylamide merged together and exhibited a broad band at 1651 cm^{-1}. The signal for N-H stretching was observed as a broad band at 3435 cm^{-1} and the C-H stretching signal was observed at 2926 cm^{-1}. Thus ^{1}H NMR and FTIR spectroscopic studies supported the formation of polyacrylamide.

In ^{1}H NMR of β-CD-PAM, a peak at 1.51 ppm was due to $–_{CH2}$ proton signals of polyacrylamide and a peak at 2.07 ppm has been attributed to –CH proton signal of PAM. A broad peak at region of 3.44 to 3.52 ppm was due to C_2, C_4 protons of β-CD. Another broad band in the region of 3.73 ppm to 3.82 ppm has been attributed to C_3, C_5 and C_6 proton signals. The signal at 4.92 ppm is due to anomeric C_1 proton signal. The FTIR spectrum also supported the formation of β-CD-PAM through its characteristic bands like 3444 cm^{-1} for O-H and N-H stretching, 1661cm^{-1} for both C=O stretching and N-H bending and a signal in the range of 1125 cm^{-1} due to ether linkages in β-CD.

The synthesis of 3-β-CD-PAM was also confirmed by the presence of both PAM and 3-β-CD ^{1}H NMR signals. The signal of aromatic protons of benzene ring in β-CD trimer was observed at 7.65 ppm and the triazole ring protons were observed at 8.54 ppm. In case of FTIR study of 3-β-CD-PAM, along with general signals of PAM and β-CD units, the characteristic C=O stretching of triprop-2-ynyl benzene-1,3,5-tricarboxylate core used in click reaction was observed at 1709 cm^{-1} and the C=C stretching of benzene ring mixed with C=O stretching and N-H bending of amide of PAM and observed at 1651 cm^{-1}. Thus the ^{1}H NMR and FTIR spectroscopic studies confirmed the formation of PAM, β-CD-PAM and 3-β-CD-PAM.

Molecular weights determination

An Ubbelohde viscometer with a capillary diameter of 0.58 mm (CS/S: 0.00386) at 25 ± 0.1 °C was used for viscosity measurement of the aqueous solutions of polymers. The time of flow for solutions was measured at four different concentrations (1%, 0.5%, 0.25%, and 0.125%). The intrinsic viscosity of the polymers was determined by extrapolating the plots of inherent viscosity versus concentration (η_{inh} vs. C) and reduced viscosity versus concentration (η_{red} vs. C) to zero concentration. The point of intersection of these plots gave the value of intrinsic viscosity. The molecular weights of PAM, β-CD-PAM and 3-β-CD-PAM were evaluated by employing their respective intrinsic viscosities in Mark Houwink equation, $[\eta] = K[M]^{\alpha}$ where $[\eta]$ is intrinsic viscosity, K and α are constants and M is molecular weight of the polymer. The intrinsic viscosities of the polymers calculated and the corresponding molecular weights were tabulated in **Table S1**. It was observed that PAM had highest intrinsic viscosity and thus maximum molecular weight than β-CD-PAM and 3-β-CD-PAM. This can be explained by the fact that, as relatively more amount of acrylamide was used for the synthesis of PAM, linearly longer chains of PAM were expected and thus they had close packing which resembled in its high intrinsic viscosity. In order to synthesize the star polymers (β-CD-PAM and 3-β-CD-PAM) relatively less amounts of acrylamide were used and thus shorter PAM chains were expected. Moreover, the star polymers do not have much close packing due to the presence of branches and thus intrinsic viscosity decreases. When β-CD-PAM is compared with 3-β-CD-PAM, the later one had less intrinsic viscosity due to presence of much more branching than the former one.

Table S1. Intrinsic viscosities and molecular weights of PAM, β-CD-PAM and 3-β-CD-PAM.

Polymer	Intrinsic Viscosity [η] in dL/g	Molecular weight (Da)	K	α
PAM	0.937	1.64×10^5	6.31×10^{-5}	0.80
β-CD-PAM	0.846	1.44×10^5	6.31×10^{-5}	0.80
3-β-CD-PAM	0.810	1.36×10^5	6.31×10^{-5}	0.80

Procedure to plot calibration curve of drug

By using the 0.1 mg/mL stock solution of drug in PBS, 0.08, 0.06, 0.04, 0.02, 0.04, 0.02, 0.01, 0.008, 0.006, 0.004, 0.002 and 0.001 mg/mL solutions were prepared up on dilution. The characteristic absorbance peak of the drug (at 276 and 485 nm for DS and DOX, respectively) was observed by using a UV-Visible Spectrophotometer. Then, the calibration graph was plotted between concentration of the drug and absorbance.

Fluorescence spectroscopy

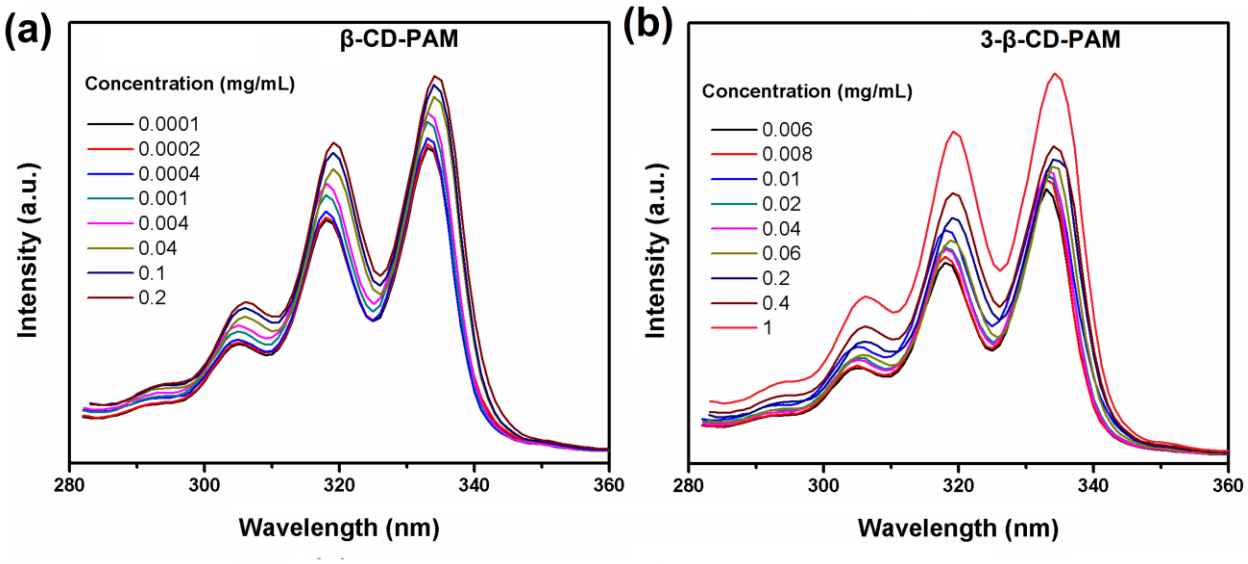


Fig. S2. Fluorescence excitation spectra (λ_{em} = 390 nm) of pyrene (6×10^{-7} M) in the presence of progressively higher concentration (C) (mg/mL) of **(a)** β-CD-PAM and **(b)** 3-β-CD-PAM.

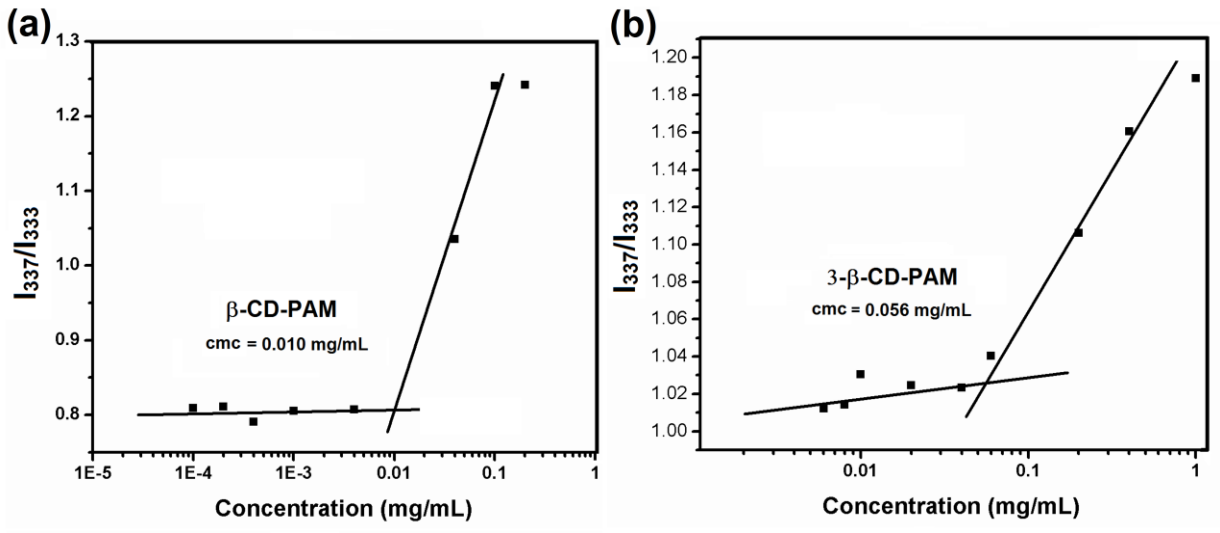


Fig. S3. Semi logarithmic plot of the fluorescence excitation intensity ratio (I_{337}/I_{333}) of pyrene (6 × 10^{-7} M) (monitored at λ_{em} = 390 nm) vs the concentration of (a) β-CD-PAM and (b) 3-β-CD-PAM, in water.

Due to the presence of β-CD cores and hydrophilic PAM parts in β-CD-PAM and 3-β-CD-PAM that could form nanostructured aggregates by means of their self assembly in aqueous solutions. This aggregation might be because of hydrogen bonding between the secondary alcoholic functionalities present on β-CDs and the intermolecular hydrogen bonding among PAM units. Fluorescence spectroscopy was used to determine the critical micelle concentration (cmc) of both star polymers using pyrene as the probe. β-CD-PAM and 3-β-CD-PAM were observed to have cmc at 0.010 and 0.056 mg/mL respectively **(Fig. S3).**

Due to presence of more amount of hydrophobic parts (β-CD) in 3-β-CD-PAM, the tendency of self aggregation was greater than β-CD-PAM. Thus the cmc value of 3-β-CD-PAM was greater than that of β-CD-PAM. In case of β-CD-PAM, the hydrophilic parts had much domination over

hydrophobic parts and thus aggregation became difficult. So, β-CD-PAM could form only small aggregates. Similar results were also observed in DLS study.

Scanning electron microscopy

SEM images of the polymers **(Fig. S4)** were recorded by the scanning electron microscope (HRSEM SUPRA 40, ZEISS (Germany)).

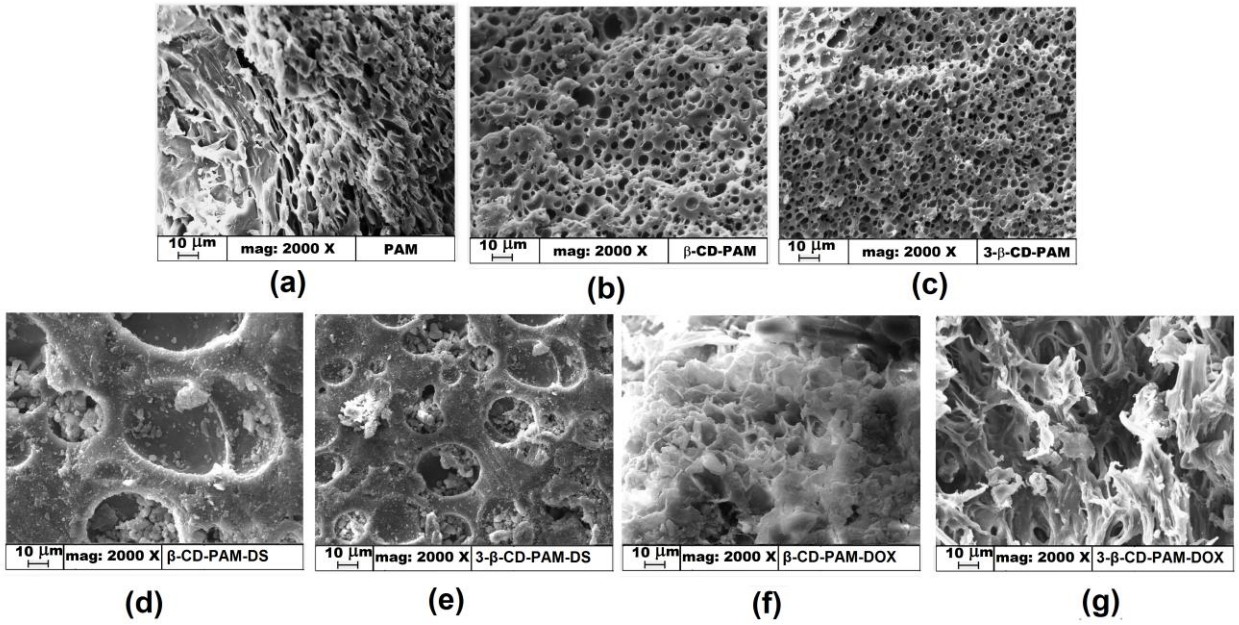


Fig. S4. The images from a to g represent the scanning electron micrographs of PAM, β-CD-PAM, 3-β-CD-PAM, β-CD-PAM-DS, 3-β-CD-PAM-DS, β-CD-PAM-DOX and 3-β-CD-PAMDOX respectively.

The micrographs revealed that PAM had rough surface. The surface morphology of β-CD-PAM exhibited its porous nature due to the presence of β-CD cores and smaller polyacrylamide chains. In case of 3-β-CD-PAM, the number of pores in unit area was observed to be more than β-CD-

PAM resembling the presence of more number of β-CD moieties. In case drug loaded polymers, DS loaded β-CD-PAM and 3-β-CD-PAM had similar morphologies which differed from the parental polymers. DOX loaded β-CD-PAM and 3-β-CD-PAM also had similar morphologies which differed from the parental polymers ads well as DS loaded β-CD-PAM and 3-β-CD-PAM. Thus change morphologies after loading the drug supports the loading of drug.