# **Electronic Supplementary Information**

# Synthesis of Calixarene-Polyglycerol Conjugates and their Self-Assembly Toward Nano and Microtubes

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#### **EXPERIMENTAL**

#### Materials and Methods

Sodium methoxide, methanol, 1,4 dioxan acetone, deuterium oxide, and citric acid and Ion exchange IV (weakly acidic cation exchange) were purchased from Merck. Glycidol, curcumin and dialysis bag (Mn cutoff 2000) were provided from Sigma-Aldrich (St Louis, Missouri). Infrared (IR) spectra were obtained using a Nicolet 320 FT-IR. <sup>1</sup>H NMR spectra were recorded in D<sub>2</sub>O on a Bruker DRX 400 (400MHz). Ultraviolet (UV) spectra were recorded on a Shimadzu (1650 PC) scanning spectrophotometer. Ultrasonic bath (Model: 5RS, 22 KHZ, Made in Italy) was used to disperse materials in solvents. The particle size and zeta potential of materials were determined using dynamic light scattering (DLS) (zetasizer ZS, Malvern Instruments). Surface imaging studies were performed using atomic force microscopy (AFM) in ambient condition and on silicon surface. Morphology and size of selfassemblies were investigated by scanning electron microscopy (SEM; MIRA3 TESCAN, Czech). For the SEM observations, copolymers were dissolved in water (0.1 mg/mL) and then a drop of the prepared solutions was spread on a substrate. The substrate was left at for 24 and 3 days and then they were dried at 40 °C over night. The room temperature prepared solid samples for the recording of images were coated by a thin layer of the gold.

# Preparation of Calix-PG

The Calix-PG was synthesized via an anionic ring opening multibranching polymerization (ROMBP). Briefly sulfonatocalix[4]arene (Calix) (0.1 g, $1.34 \times 10^{-4}$  mol) was added to a solution of sodium methoxide in dry methanol (0.075g, $1.38 \times 10^{-4}$  mol) (3 mL suspension) and mixture was added to a polymerization ampoule equipped with a magnetic stirrer under nitrogen atmosphere for 2 h at room temperature for partial deprotonation of P-

sulfonatocalix[4]arene. Then methanol was vaporized using vacuum oven for 2 h at 70 °C. Glycidol (2.5 mL) was added over a period of 3 h using a syringe pump and reaction continued for additional 24 h. Then the product was cooled and dissolved in methanol, and neutralized by passing three times through a column packed with cation exchange resin. The polymer was then precipitated into acetone and stirred for 2 h. The product was dried using vacuum oven at 80 °C. The final product is a yellow and viscous compound.

In the IR spectrum(Figure S1) of calix-PG three absorbance bands assigned to O-H,C-O-C,C-H groups are appeared at 3332,1115 and 2877 cm<sup>-1</sup> respectively. Absorbance band at 1265 cm<sup>-1</sup> is due to the S=O bonds of sulfonato groups of calixarene and confirm conjugation of polyglycerol to calixarene.

In the <sup>1</sup>H NMR spectrum of Calix-PG (Figure S2 ) signals at 3.4 -3.5 ppm are corresponded to the C-H protons of polyglycerol. In the <sup>13</sup>C NMR spectrum of Calix-PG (Figure 2) signals at 60-79 ppm are assigned to the polyglycerol carbons.



Figure 1S. IR spectra for a) Calixarene, b) Calix-PG and c) Calix-PG-PCA.



Figure 2S. a) The <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Calix-PG in  $D_2O$ .

# Preparation of Calix-PG-PCA

In a two necked round bottom flask, Calix-PG (0.13 gr) was added to dry methanol (1 ml). Then citric acid (0.8 g) dissolved in dry 1,4 dioxane (25 ml) with dispersed blue silica gel was added to above solution in an oil bath and mixture was heated up to 95 °C. Mixture was stirred for addition 24 h at this temperature. The product of the reaction was collected by filtration and then precipitation in acetone and dialysis against water for 48 h.

In the IR spectrum (Figure 1) of Calix-PG-PCA five absorbance bands at 2700-3600 and 1710 cm<sup>-1</sup> are assigned to the hydroxyl and carbonyl groups of citric acid block. Additional peak at 1150 is corresponded to etheric bonds of polyglycerol backbone.

In the <sup>1</sup>H NMR spectra of Calix-PG-PCA, signals of methylene groups of citric acid segments can be observed at 1.8-2.7 ppm and signals for PG are appeared at 3.2-4.7 ppm. In the <sup>13</sup>C NMR spectra, signals at 42 and 174 ppm are assigned to the carbons of citric acid unites. Signal at 174 is assigned to the carbonyl groups of PCA. In the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Calix-PG-PCA signals of PG backbone and calixarene core are determined on the spectra (Figure 3S).



Figure 3S. <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra of Calix-PG-PCA in D<sub>2</sub>O.

#### Dynamic Light Scattering (DLS)

Size of copolymers in the fresh samples (before assembly) were determined using dynamic light scattering (DLS) (zetasizer ZS, Malvern Instruments). DLS diagrams show 40 and 62 nm sizes for Calix-PG and Calix-PG-PCA in water respectively (Figures 4S and 5S).



Figure 4S. DLS diagram of fresh sample of Calix-PG in water.



Figure 5S. DLS diagram of fresh sample of Calix-PG-PCA in water.

### The preparation of samples for the AFM studies

Polymer of copolymers (0.001g) were dissolved in 2.5 mL doubly distilled water. Solution was for certain times to ensure self-assembly of polymer. Then a drop of the prepared solutions was plummeted onto the holder (silica for AFM). Upon the evaporation of the solvent at room temperature, solid samples for the demoing of images were acquired.

#### Studies with an optical microscope

The copolymers (0.001g) were dissolved in 2.5mL doubly distilled water. And after two days were examined by light microscopy confirmed the self-assembly of polymers



**Figure 6S.** Optical Microscopy images of the wall of microtubes produced by self-assembly of Calix-PG-PCA copolymer after 2 days.



**Figure 7S.** AFM image of the wall of microtubes produced by self-assembly of Calix-PG-PCA copolymer after 3 days.



**Figure 8S.** AFM images and height profiles of self-assemblies of Calix-PG-PCA copolymer after a) and b) 6 h, c-e) 24 h and f) and g) 3 days, respectively.



**Figure 98.** SEM images and height profiles of self-assemblies of a) Calix-PG after 3 days and b), c) and d) Calix-PG-PCA copolymer after 3 days, respectively.

#### UV-Vis spectral titration

For encapsulation studies, curcumin  $(2.8 \times 10-6 \text{ mol/L})$  in PBS (pH 7.4) was sonicated for five hour at room temperature and added to different concentrations of the Calix-PG ranging from  $4.6 \times 10^{-6}$  to  $6.4 \times 10^{-05}$  mol/L. The UV-vis spectrum of curcumin was recorded on a Shimadzu (1650 PC) scanning spectrophotometer. The maximum absorptions of curcumin at 261 and 448 nm were raised upon increased concentration of Calix-PG.

The apparent formation constant of the complex was obtained using Benesi- ldebrand's method (double-reciprocal plot). The experimental results were fixed presupposing the formation of 1:1 curcumin-Calix-PG and curcumin-Calix-PG-PCA complexes, according to the following reactions:

The apparent formation constants (Kf) of the complexes (for example for curcumin-Calix-PG) in these reactions would be indicated by:

K<sub>f</sub>=[ curcumin-Calix-PG ]/[ Curcumin][ Calix-PG]

where [curcumin-Calix-PG], [curcumin], and [calix-PG]<sup>1</sup> are in equilibrium. The relationship between concentration and absorption of Calix-PG are related by the following equation

$$\frac{1}{A-A_0} = \frac{1}{(A_{\infty}-A_0)K_{\!f} \; [\texttt{calix-PG}]^l} + \frac{1}{A_{\infty}-A_0}$$

In this equation, absorbance of the curcumin at each copolymers concentration tested is shown by A.  $A_0$  and  $A_{\infty}$  are absorbance of curcumine in the absence of copolymers and when the whole of the curcumin with copolymers is given complex, respectively. Thus by drawing a diagram of values 1/A-A0 vs. 1/[Calix-PG], the linear relationship is obtained that describes the stoichiometric coefficient, and subsequently the value of Kf, is acquired.

This complexation can be illustrated by the subsequenting step wise mechanism, involving the initial formation of the 1:1 host:guest complex as defined above in rxn. (1), consequently by addition of a second host to give the 2:1 host:guest complex calix-PG2:curcumin:

Calix-PG+ calix-PG:curcumin  $\frown$  [calix-PG]2:curcumin rxn (3)

These two equilibria are illustrated by the equilibrium constants *K*1 and *K*2:

K1 = [Calix-PG:curcumin]/([Calix-PG][curcumin]) rxn (4)

K2 = [Calix-PG2:curcumin]/([Calix-PG][Calix-PG:curcumin]) rxn (5)

If K2 >K1 is, then the stoichiometric coefficient will be 2:1 for curcumin to copolymers .The value K2( $7.50 \times 10^8$ ) is larger than K1( $3.30 \times 10^4$ ) that describe of ratio of 2:1 the stoichiometric coefficient for host:guest complex between curcumin and Calix-PG, and subsequently the value of K2( $6.50 \times 10^8$ ) is larger than K1( $6.10 \times 10^4$ ) that describe of ratio of 2:1 the stoichiometric coefficient for host:guest complex between curcumin and Calix-PG, PCA (Figures 9S and 10S).



**Figure 10S.** a) UV-vis spectra of polymer Calix-PG and curcumin in PBS (pH 7). b) plot with values 1/A - A0 versus 1/[Calix-PG] for determination of K1 and K2.



Figure 11S. a) UV-vis spectra of polymer Calix-PG-PCA and curcumin in PBS (pH 7). b)



plot with values 1/A - A0 versus 1/[Calix-PG-PCA] for determination of K1 and K2.

# Preparation of curcumin-loaded copolymers

Curcumin (0.01g) was dissolved in PBS and added to solution of self-assemblies of polymer or copolymer (0.001g) in PH 7.4. Mixture was sonicated at 37 °C for 5h and subsequently it was stirred overnight. The solution was centrifuged (3000 rpm) for 5 minutes. The amount of drug loading content (LE) was determined by UV-vis spectra or spectrofluorometry and concentration of curcumin was determined using calibration curve. LE of nanotubes formed

by self-assembly of Calix-PG is 41% also it is 57% for microtubes produced by Calix-PG-PCA.

DLE (wt%) = (weight of total drug – weight of free drug/weight of total drug)  $\times 100\%$ 

# Drug release study

Solution of the loaded curcumin by nano- and microtubes in PBS (pHs 7.4 and 5) was transferred to a dialysis bag (Mn cutoff 2000) and immersed in 50 ml of PBS. 5 % tween-80 was added to PBS phase and at interval times, 2 ml of the dialysate was taken out and replaced by 2 ml fresh PBS. Concentration of the released curcumin was determined by UV-vis and using calibration curve.



**Figure 12S.** Fluorescence images of curcumin encapsulated by a) Calix-PG and b) Calix-PG-PCA self-assemblies.