Supporting Information

Amphotericin B Encapsulation and Controlled Release from PSSA-b-PMAV Micelles for Anti-Leishmanial Treatment

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1. Formulae used for calculation

Using the following formulas, the various reaction parameters were calculated:

$$\%C_{NMR} = \frac{A_{Polymer}}{A_{Polymer} + A_{Monomer}} \times 100 \qquad Eq.(S1)$$

Where,

$\%C_{NMR}$ = % Conversion determined by ^{1}H NMR,

$A_{Polymer}$ = Integral of protons in polymer,

$A_{monomer}$ = Integral of protons in monomer

Monomer conversion for the kinetic studies of the polymerization reaction was calculated by ^{1}H-NMR analysis of the crude mixture which was taken out at different time intervals during the polymerization reaction, and the monomer conversion (% C) was calculated by comparing the integrated peak area of repeating unit of the polymer $A_{polymer}$ with the integrated peak area of the monomer $A_{monomer}$ by applying the equation (1),

The theoretical number average molecular weight Mn of the homopolymer PSSA was calculated using the following equation

$$M_n(theo) = \frac{[PSSA]_0}{[CTA]_0} \times ConversionNMR \times MW_{PSSA} + MW_{CTA} \qquad Eq.(S2)$$

Where M_{CTA} and M_{PSSA} are the molecular weight of Trithiocarbonate CTA and monomer; $[SSA]_0$ and $[CTA]_0$ are the initial concentration of SSA monomer and Trithiocarbonate respectively.

$$Degree\ of\ polymerization\ (DPn) = \frac{\frac{Area}{Integral} of\ PSSA \times No.\ of\ proton\ of\ BDTTC}{\frac{Area}{Integral} of\ BDTTC \times No.\ of\ proton\ of\ PSSA} \qquad \text{Eq. (S3)}$$

$$Mw = DP_n \times \text{Molecular weight of monomer} \qquad \text{Eq. (S4)}$$

Calculation of Yield of Monomer:

Theoretical yield (g) = Moles of limiting reagent × Molar mass of product

Percent yield = (Actual yield / Theoretical yield) × 100 Eq. (S5)

2. Instrumentation

The characterization of synthesized polymers was carried out using various analytical techniques. Nuclear Magnetic Resonance (NMR) spectroscopy was performed on a JEOL

Resonance ECZ 500 R spectrometer, with ^{1}H NMR recorded at 500 MHz and ^{13}C NMR at 125 MHz, using DMSO-d_6, D_2O and $CDCl_3$ as solvents at 20 °C. Fourier Transform Infrared (FT-IR) spectroscopy was conducted on a JASCO TOKYO-4700 spectrophotometer to identify characteristic functional groups, with spectra collected in the 500–4000 cm^{-1} range using potassium bromide (KBr) pellet samples. Mass spectrometry (MS) analysis was carried out using a SCIEX X500R QTOF mass spectrometer with Direct Analysis in Real Time Mass Spectrometry (DART) for rapid molecular characterization. Molecular weight analysis was performed using Gel Permeation Chromatography (GPC) on a Malvern Viscotek GPCmax system equipped with a Malvern Viscotek TDA 305 multi-detector array. The measurements were conducted in dimethylformamide (DMF) at 50 °C, with a flow rate of 1 mL/min, utilizing a T6000M General Mixed Organic Column (300 mm × 8 mm) for high-resolution separation. Calibration was performed using narrow and broad polystyrene (PS) standards due to their well-defined molecular weight distribution, solubility, and linear structure, ensuring accurate hydrodynamic volume calibration. The TDA 305 system included a refractive index (RI) detector for concentration determination, a right-angle light scattering (RALS) detector for molecular weight measurement, a low-angle light scattering (LALS) detector for enhanced molecular weight accuracy, and a viscometer detector for intrinsic viscosity analysis, collectively enabling precise polymer characterization. Thermal properties were assessed using Thermogravimetric Analysis (TGA) and Differential Scanning Calorimetry (DSC). TGA was performed on a METTLER-TOLEDO instrument (Germany) to evaluate the thermal stability of PSS macro-CTA and the PSS-b-PMAV block copolymer. Measurements were carried out from 30 to 800 °C at a heating rate of 10 °C/min under a nitrogen atmosphere. DSC was used to determine the glass transition temperature (Tg) of polymer samples, employing a METTLER-TOLEDO DSC 822 instrument (Germany). The temperature range for DSC analysis was set from 25 to 200 °C, with a heating rate of 10 °C/min. The instrument was calibrated using indium as a standard reference. The crystalline nature of RAFT-CTA, PSS, PMAV, and PSS-b-PMAV was investigated through Powder X-ray Diffraction (p-XRD) using an 18 kW Cu-rotating anode Rigaku diffractometer. Scattering data were collected over a 2θ range of 10°–80° with a scanning rate of 2°/min. Dynamic Light Scattering (DLS) measurements were conducted using a Malvern Zetasizer Ultra-series particle size analyser to determine the particle size distribution of PSS and PSS-b-PMAV. Transmission Electron Microscopy (TEM) analysis was carried out using an FEI TECHNAI G2 20 TWIN microscope operating at 200 kV to investigate the micellar morphology of the block copolymer. For TEM imaging, the polymer

was dissolved in ethanol, drop-cast onto a carbon-coated copper grid, and dried. To enhance contrast between polymer domains, selective staining was performed using osmium tetroxide (OsO_4) before imaging. SEM and EDAX experiment were performed on Thermo fisher, QUANTA 200 dried powder sample of polymer quoted on aluminum was used for analysis and UV spectra were recorded using an Agilent, USA Model-Cary (60 UV-Vis) spectrophotometer, and Fluorescence spectra were obtained with a Fluorolog FL-3-11 (Horiba Jobin-Yvon) spectrophotometer.

Table S1 RAFT polymerizations of SSA homopolymer was conducted with a monomer-to-RAFT agent-to-initiator ratio of SSA: BDTTC: AIBN = 200: 1: 0.2 at 70°C.

S.N.	Time (h)	% Conversion	M_{nTheo} (g/mol)	M_{nGPC} (g/mol)	$\ln[M]_0/[M]_t$	PDI
1	1	18	18,794	21600	0.19845	1.18
2	3	30	23,742	24200	0.35667	1.16
3	5	40	25629	26800	0.51083	1.08
4	6	43.8	29136	29450	0.57625	1.23
5	7	52	31330	31640	0.73397	1.27
6	9	58.6	34068	34870	0.88189	1.07
7	11	65	36423	36673	1.04982	1.21
8	17	77.4	41,730	42110	1.48722	1.25
9	19	79.8	44,662	44,900	1.59949	1.30

Table S2 Effect of Temperature Variation on Reaction Kinetics of SSA with BDTTC RAFT agent

Temperature(K)	k_{app} (min^{-1})	Run	Time (h)	% Conversion	$ln[M]_0/[M]_t$
333K	1.54×10^{-3}	1	1	9	0.094
		2	4	21	0.2357
		3	8	28	0.3285
		4	10	34	0.4155
		5	14	40	0.5108
		6	18	48	0.6539
		7	22	54	0.7765
343K	2.14×10^{-3}	1	1	18	0.19845
		2	4	30	0.35667
		3	8	40	0.51083
		4	10	43.8	0.57625
		5	14	52	0.73397
		6	18	58.6	0.88189
		7	22	65	1.04982
353K	2.94×10^{-3}	1	1	19.5	0.214
		2	4	31.8	0.3828
		3	8	46.5	0.6278
		4	10	53.5	0.7651
		5	14	63	1.0216
		6	18	72.5	1.2909
		7	22	78.8	1.5506

Table S3. Kinetics of Block Polymer at 80°C.

S.No.	Time (h)	% Conversion	$\ln[M]_0/[M]_t$
1	0	0	0
2	1	24.5	0.281
3	3	36	0.4462
4	5	45	0.5978
5	6	48.5	0.6635
6	7	52.5	0.7444
7	9	58.6	0.8818
8	11	64	1.0216
9	17	76.6	1.4524
10	19	79.6	1.5896

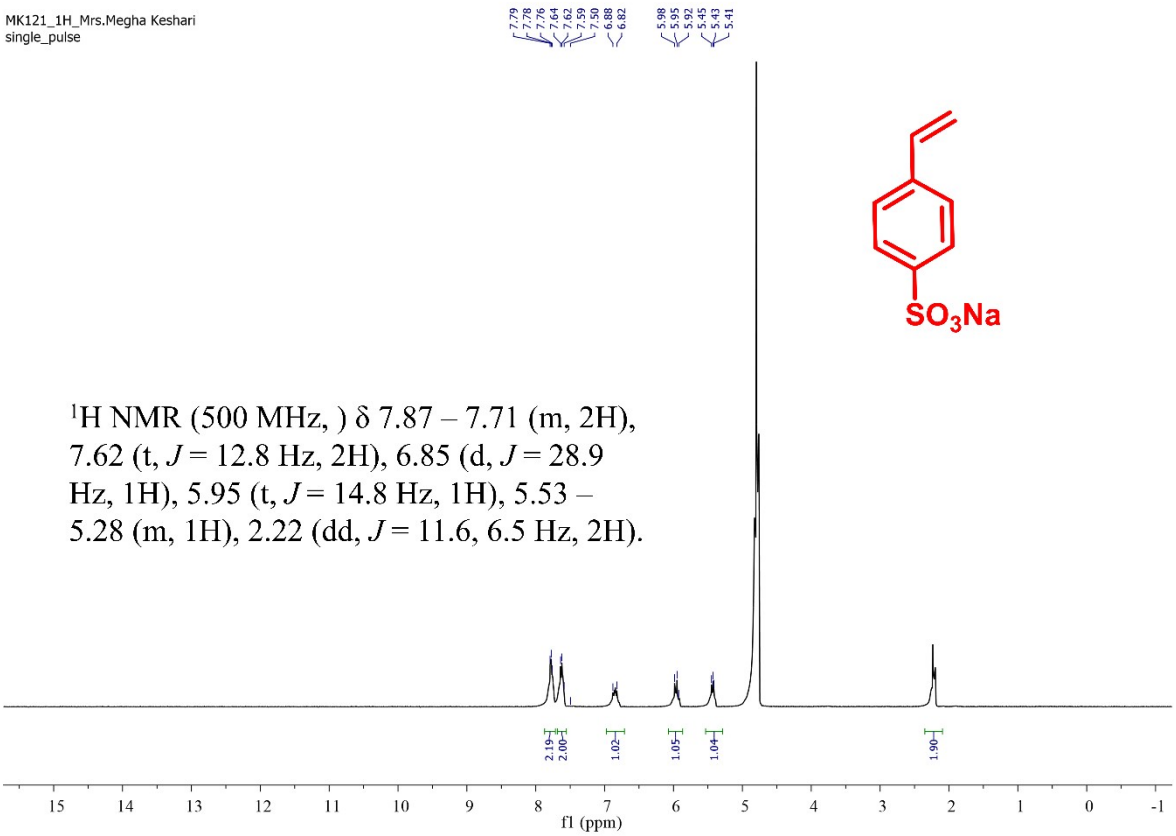


Fig. S1 ^{1}H NMR Spectrum of SSA in D_20

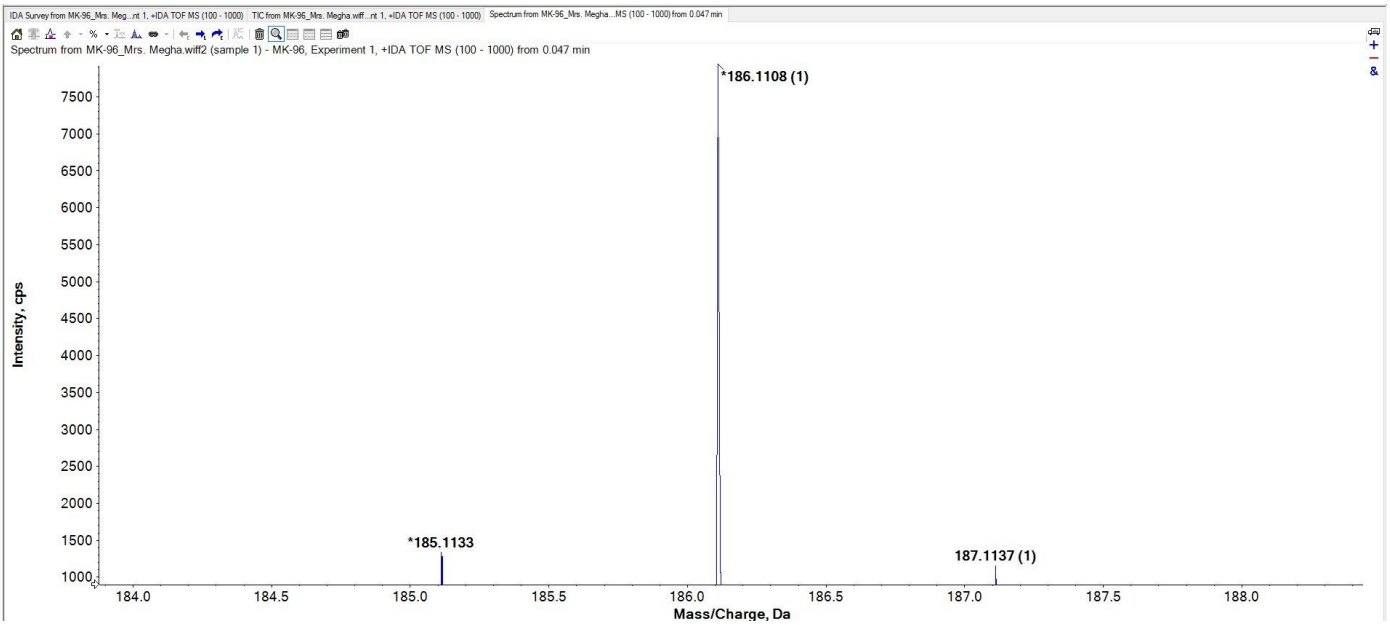


Fig. S2 HRMS analysis of synthesized Methacryloyl Valine monomer

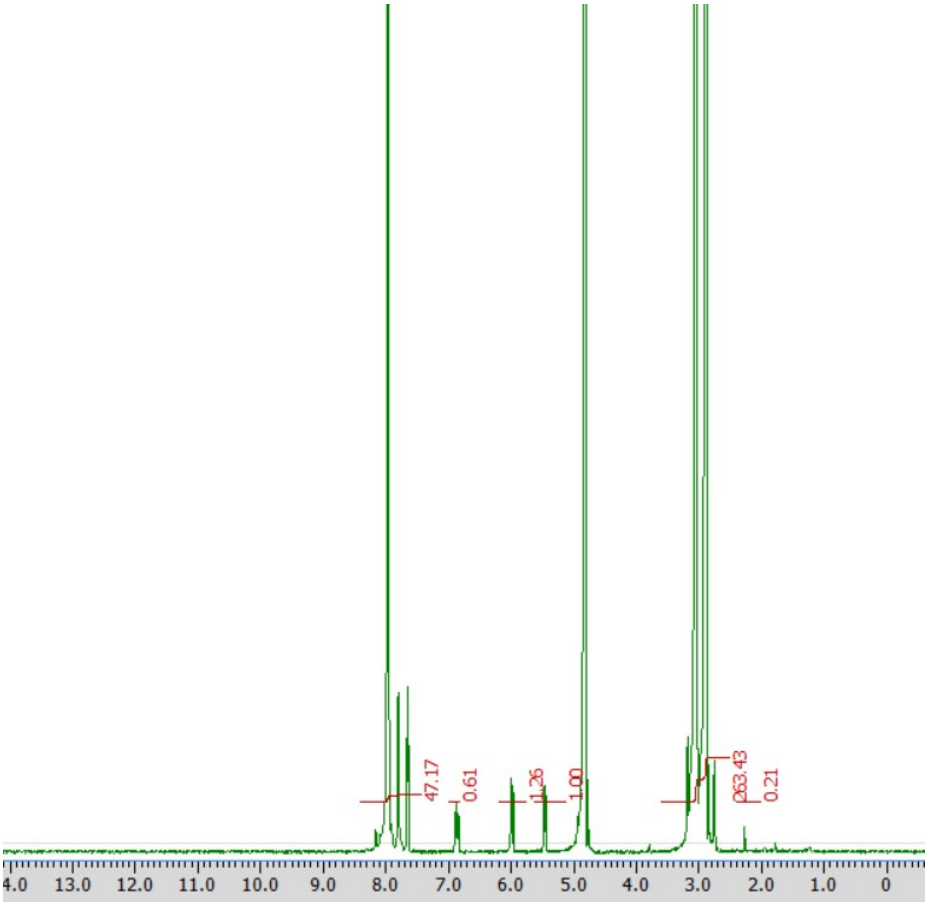


Fig. S3 ^{1}H NMR Spectrum of crude PSSA in D_2O showing 37.8 % conversion at 70˚C

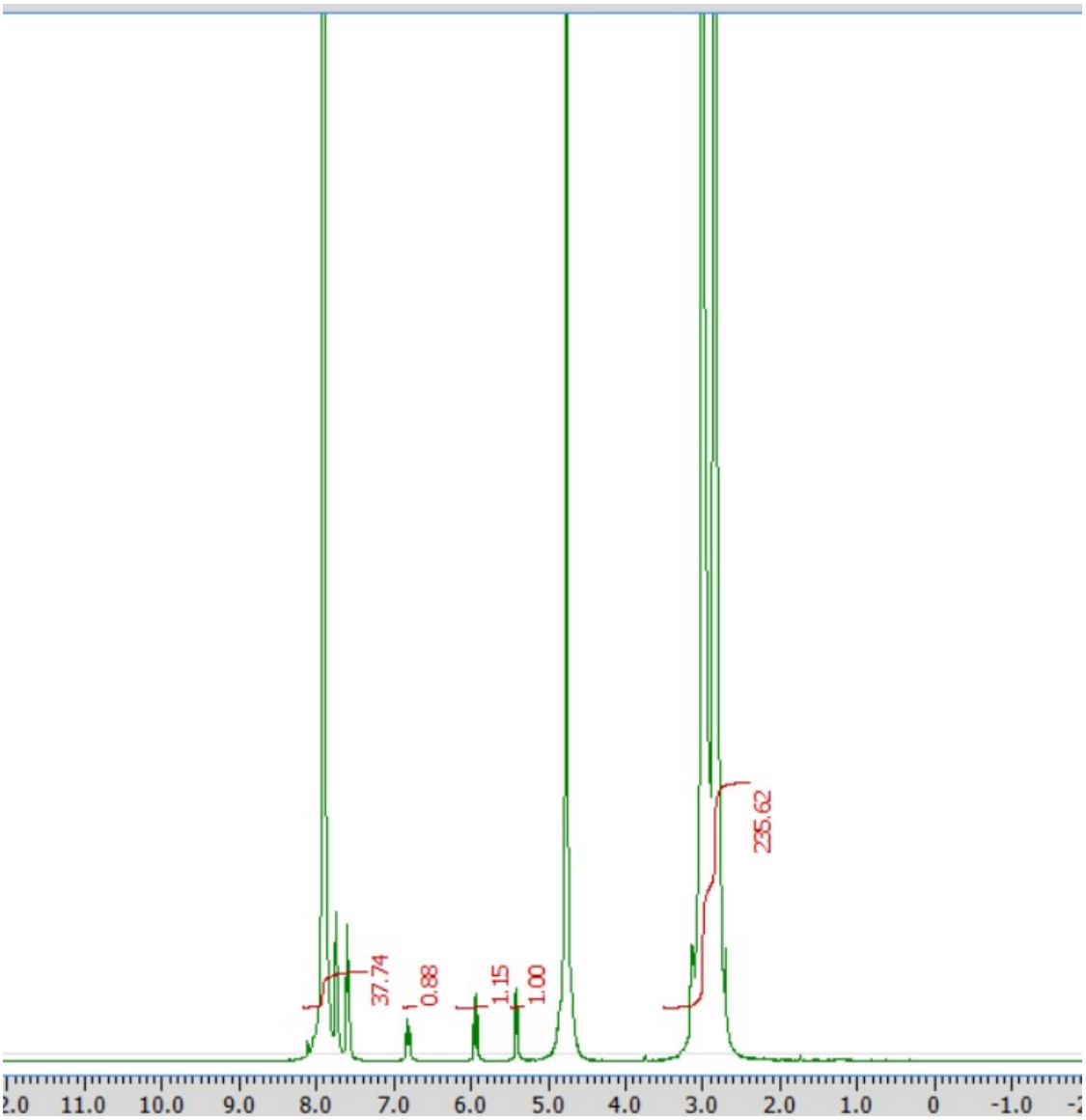


Fig. S4 ^{1}H NMR Spectrum of crude PSSA in D_2O showing 46.8 % conversion at 70°C

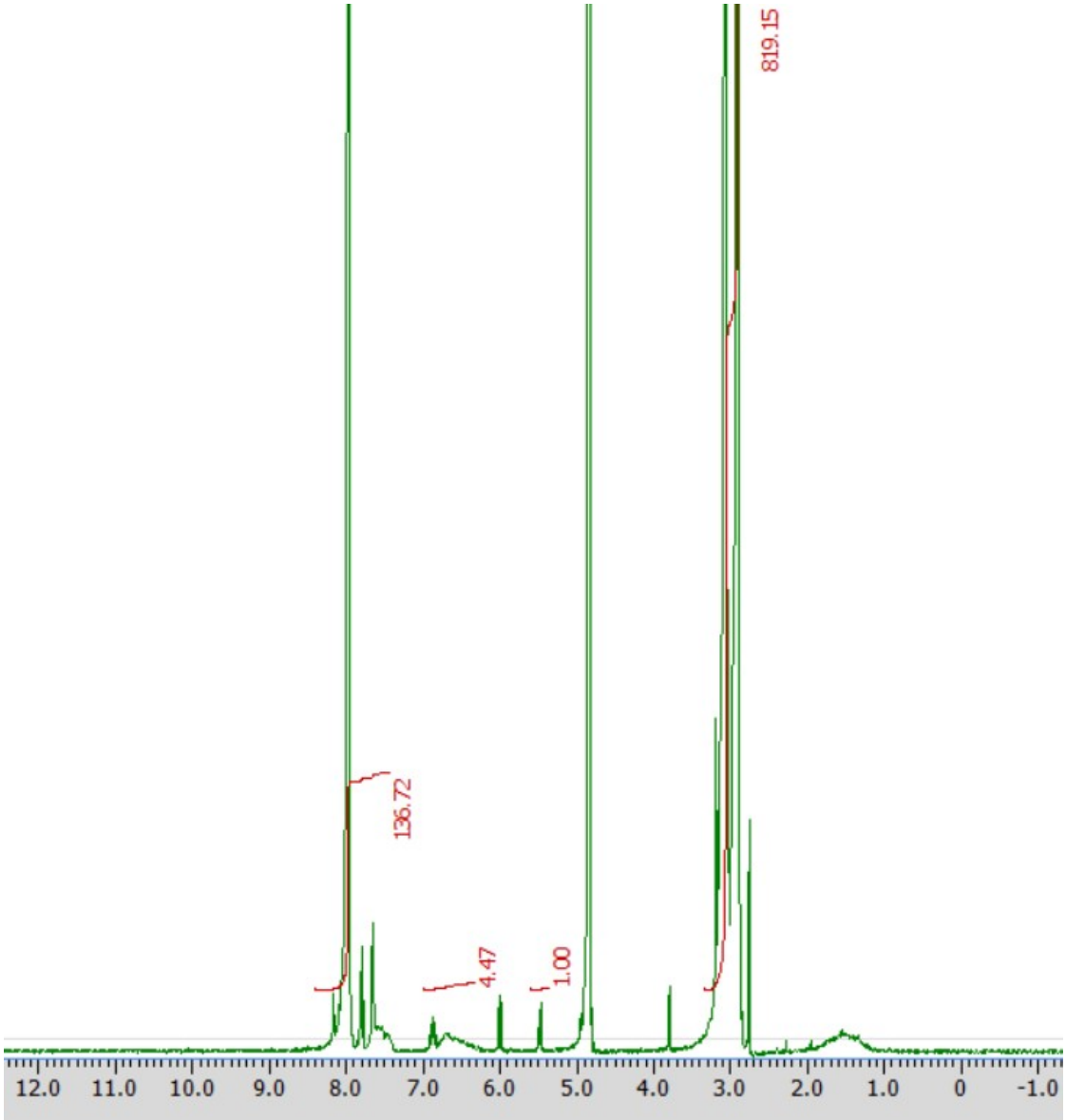


Fig. S5 ^{1}H NMR Spectrum of crude PSSA in D_2O showing 81.7 % conversion at 70°C

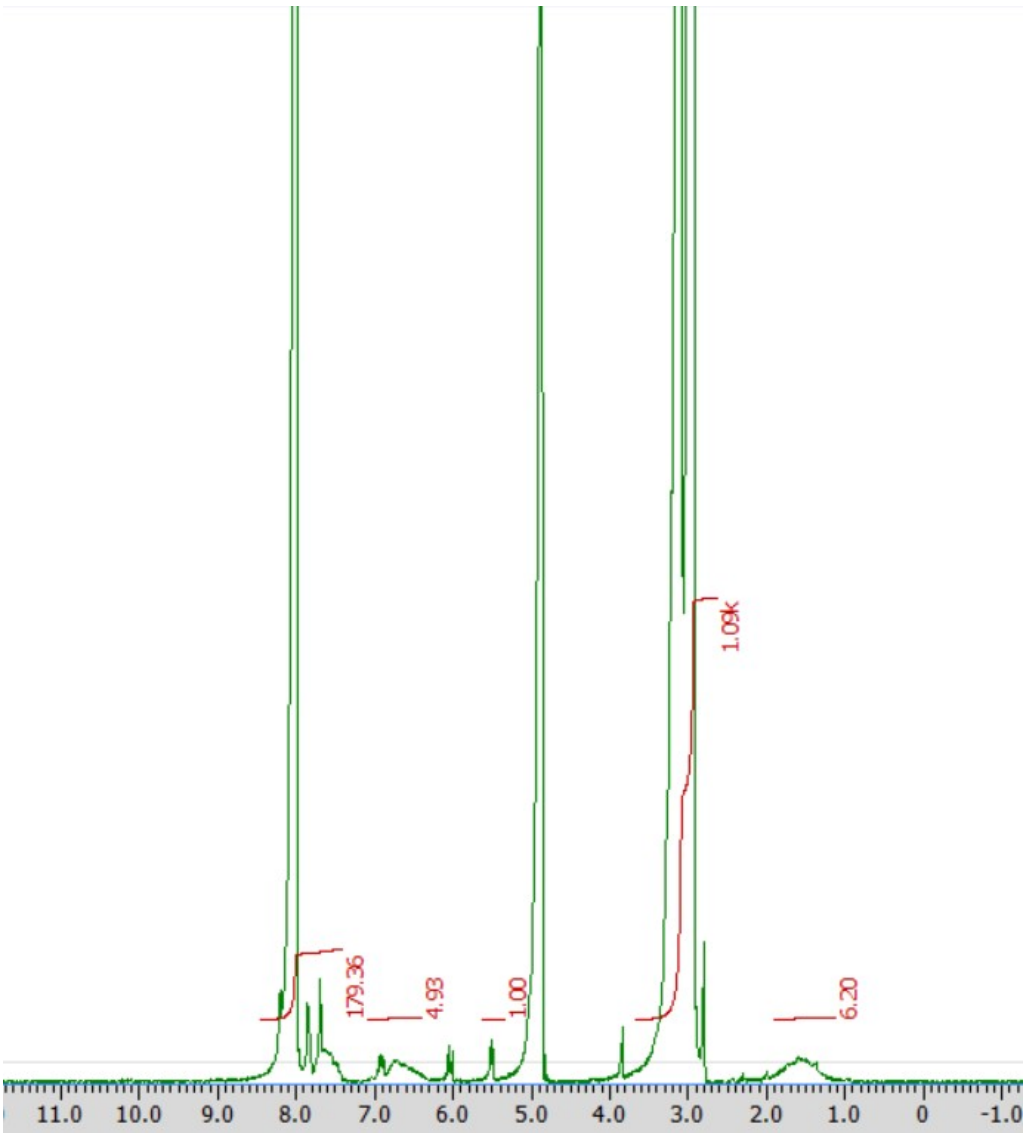


Fig. S6 ^{1}H NMR Spectrum of crude PSSA in D_2O showing 83.1 % conversion at 70°C

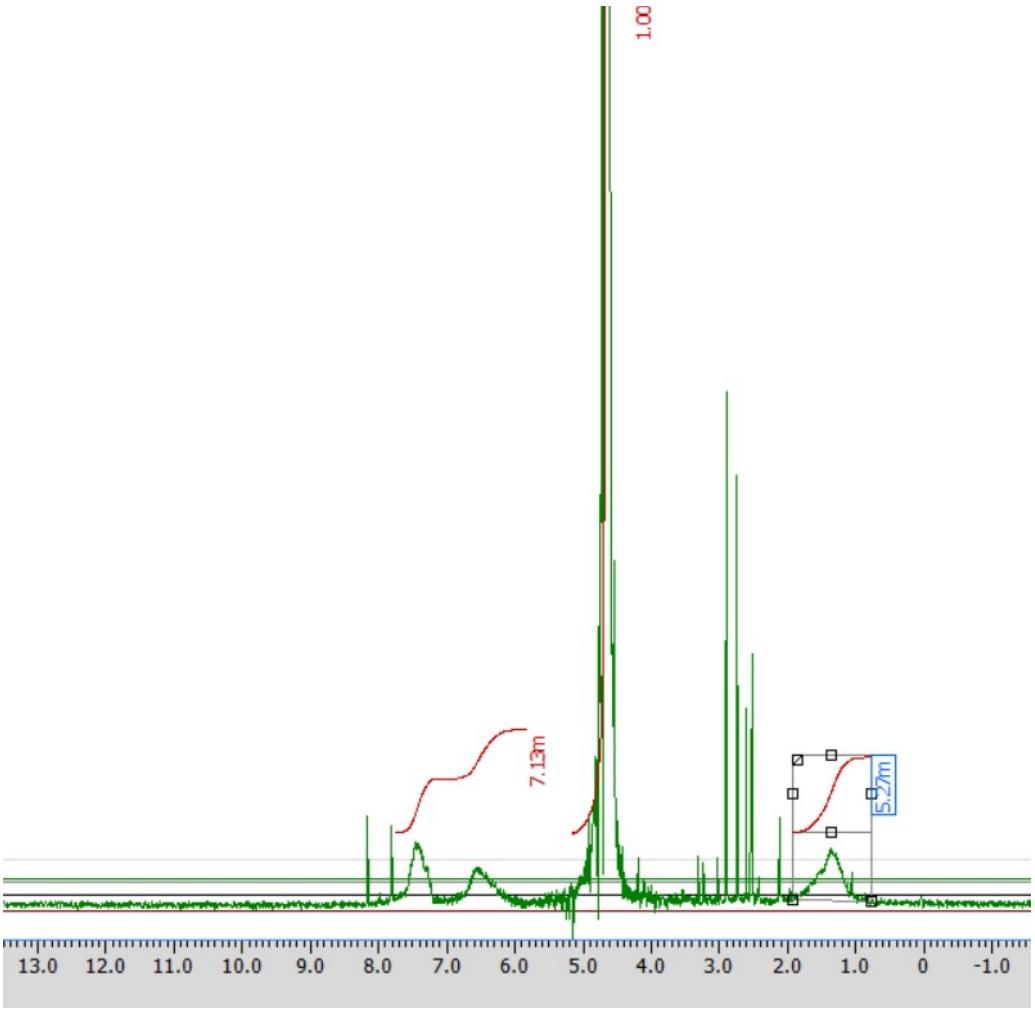


Fig. S7 ^{1}H NMR Spectrum of crude PSSA-b-PMAV block co-polymer in DMSO d_6 showing 78% conversion at 80°C.

Table S4. Molecular characteristics of the PSSA-b-PMAV block copolymer synthesized via RAFT polymerisation.

Polymer	Monomer conversion (%)	Mn (theoretical) (g/mol)	Mn (GPC) (g/mol)	D	Characterization Techniques
PSSA-macro CTA	40	25629	26800	1.08	^{1}H NMR, GPC
PSSA-b-PMAV	78	45,500	46000	1.30	^{1}H NMR, GPC

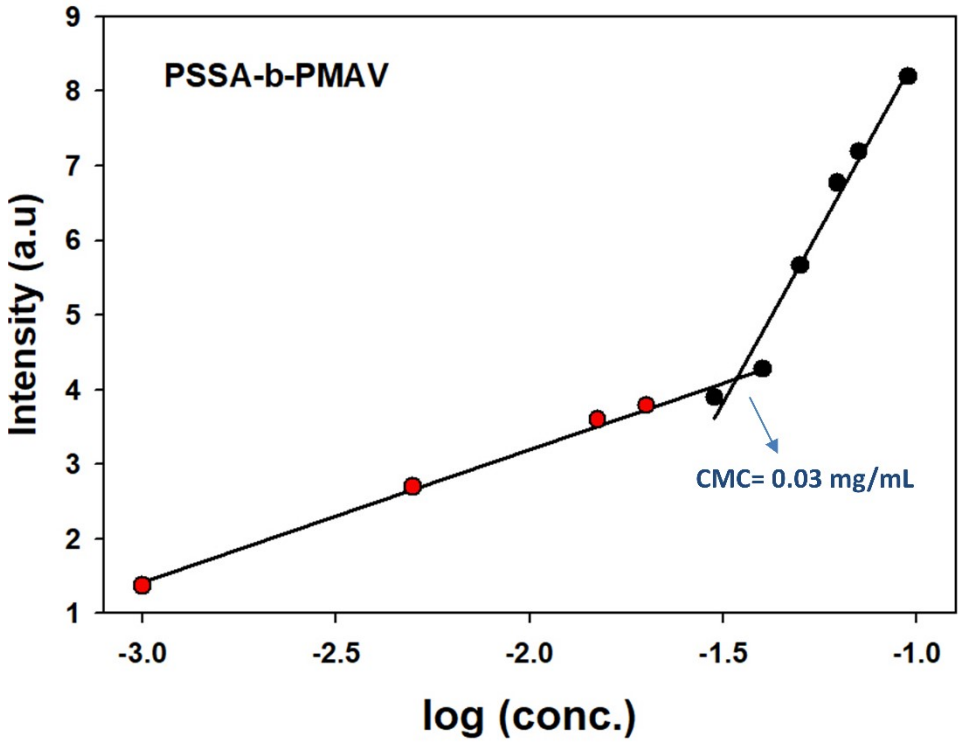
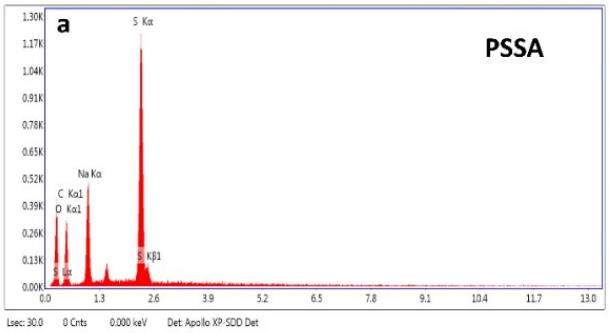
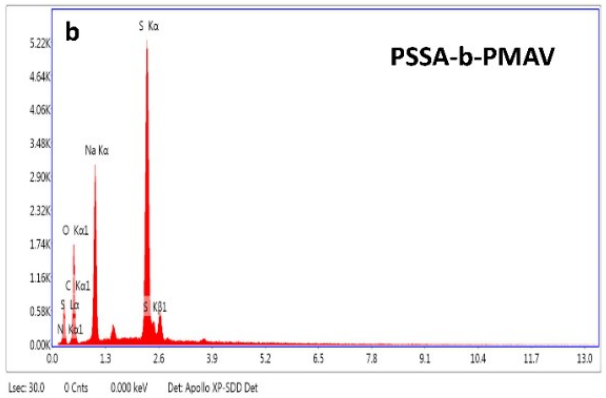


Fig. S8 CMC plot of block copolymer PSSA-b-PMAV in water (CMC=0.03 mg/mL).



Element	Weight %	Atomic %	Net Int.	Error %
C K	53.1	66.2	84.7	11.2
O K	21.7	20.3	61.9	12.8
Na K	9.6	6.3	111.2	8.6
S K	15.6	7.3	366.7	2.6



Element	Weight %	Atomic %	Net Int.	Error %
C K	35.1	48.5	155.1	11.1
N K	0.2	0.3	0.7	12.9
O K	27.5	28.5	346.8	10.4
Na K	17.1	12.3	704.9	7.2
S K	20.2	10.4	1633.2	2.4

Fig. S9 EDX analysis with elemental composition of (a) PSSA and (b) PSSA-b-PMAV

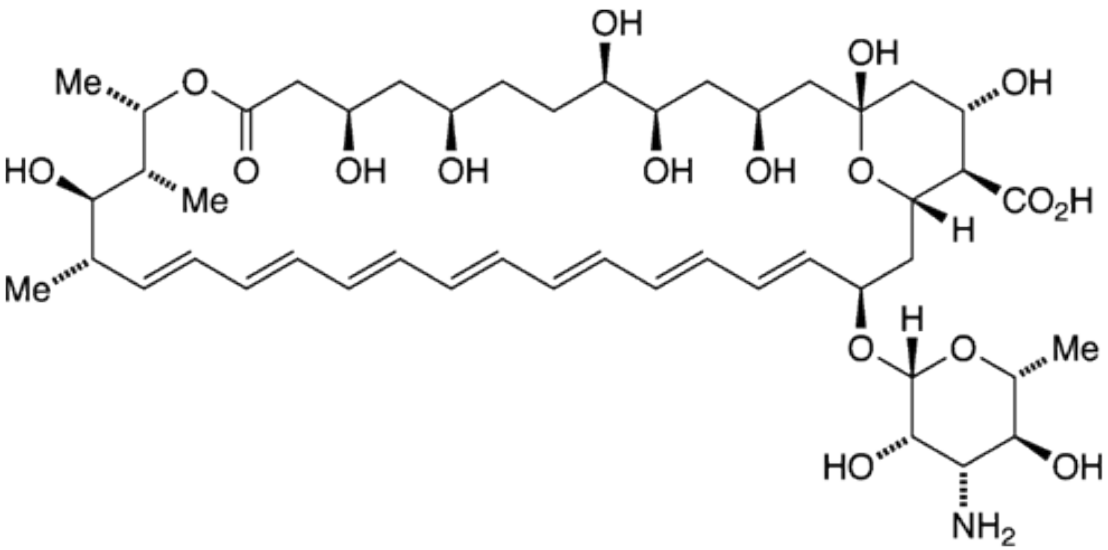


Fig. S10 Structure of Amphotericin B