Poly(methacrylic acid) complexation of amphotericin B

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Supplementary experimental information

Preparation of AmB-PMAA complexes from PMOSu

Poly(*N*-methacryloxysuccinimide)[1] (PMOSu) **4** (60 mg, 0.3 mmol of the repeat unit) was dissolved in stirred DMSO (1.2 mL) overnight in a 50 mL round bottom flask. To this stirred solution was added a solution of AmB (48 mg, 0.05 mmol) in DMSO (1.4 mL). After 2 min, an aqueous solution of sodium hydroxide (0.656 mL 1 M NaOH and 110 µL H_2O) was added dropwise to the PMOSu/AmB solution followed by the immediate addition of pure water (6.7 mL). The resulting solution was left to stir at room temperature for 1 h. Uncomplexed AmB was then removed by 24 h dialysis against 1 L of pure water using a Visking dialysis membrane (MW12-14 kDa, Medicell International). During dialysis, the dialysate was changed six times. The dialysed solution of the complex was filtered using a 0.2 µm Minisart® syringe filter (Fisher, Cat. No. FDP-620-030U) and then freeze-dried to afford a red coloured powder (mass 96.7 mg). Samples were protected from light by wrapping in aluminium foil at all times during preparation and storage.

Reference

(1) A. Godwin, M. Hartenstein, A. Müller and S. Brocchini, *Angew. Chem. Int. Ed. Engl.* 2001, **40**(3), 594-597.

Supplementary Figures

Figure S1

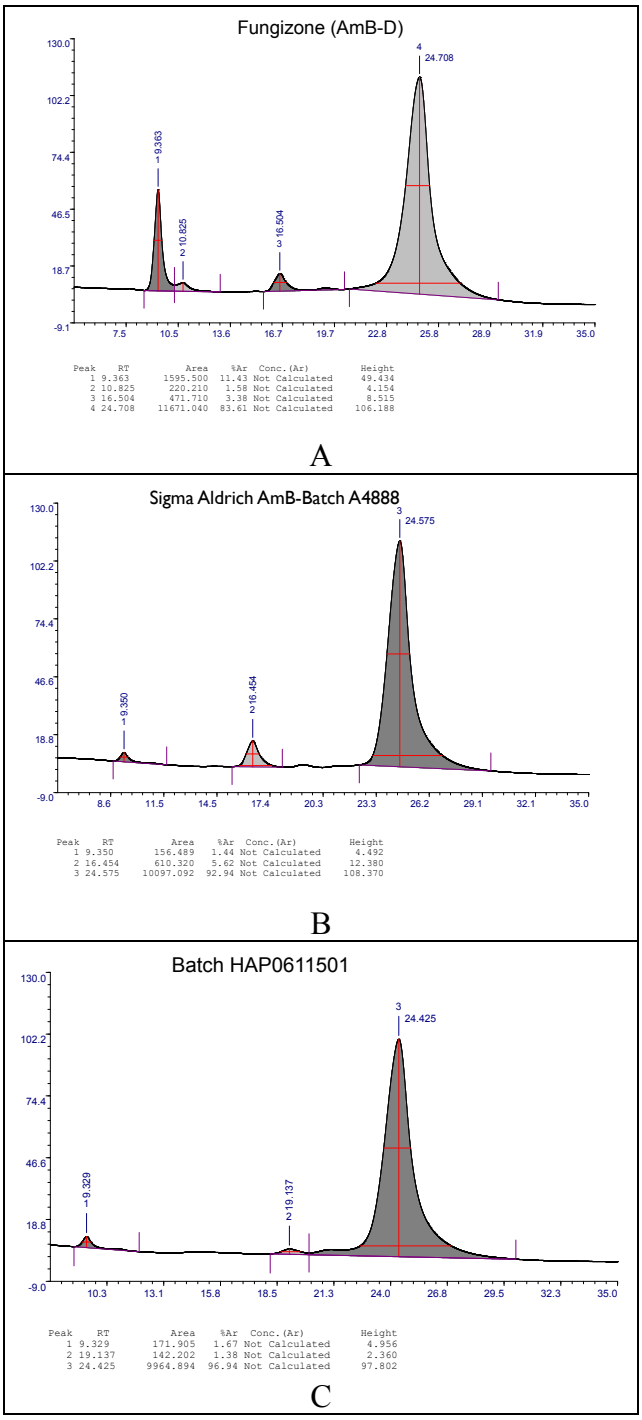


Figure S1. The HPLC chromatogram for Funigzone (AmB-D) (A) was obtained using the amount of AmB-D necessary to have a 60 μg/mL concentration of AmB and the chromatograms for (B) and (C) were obtained using 60 μg/mL of material. AmB eluted at ~24.5 minutes.

Figure S2

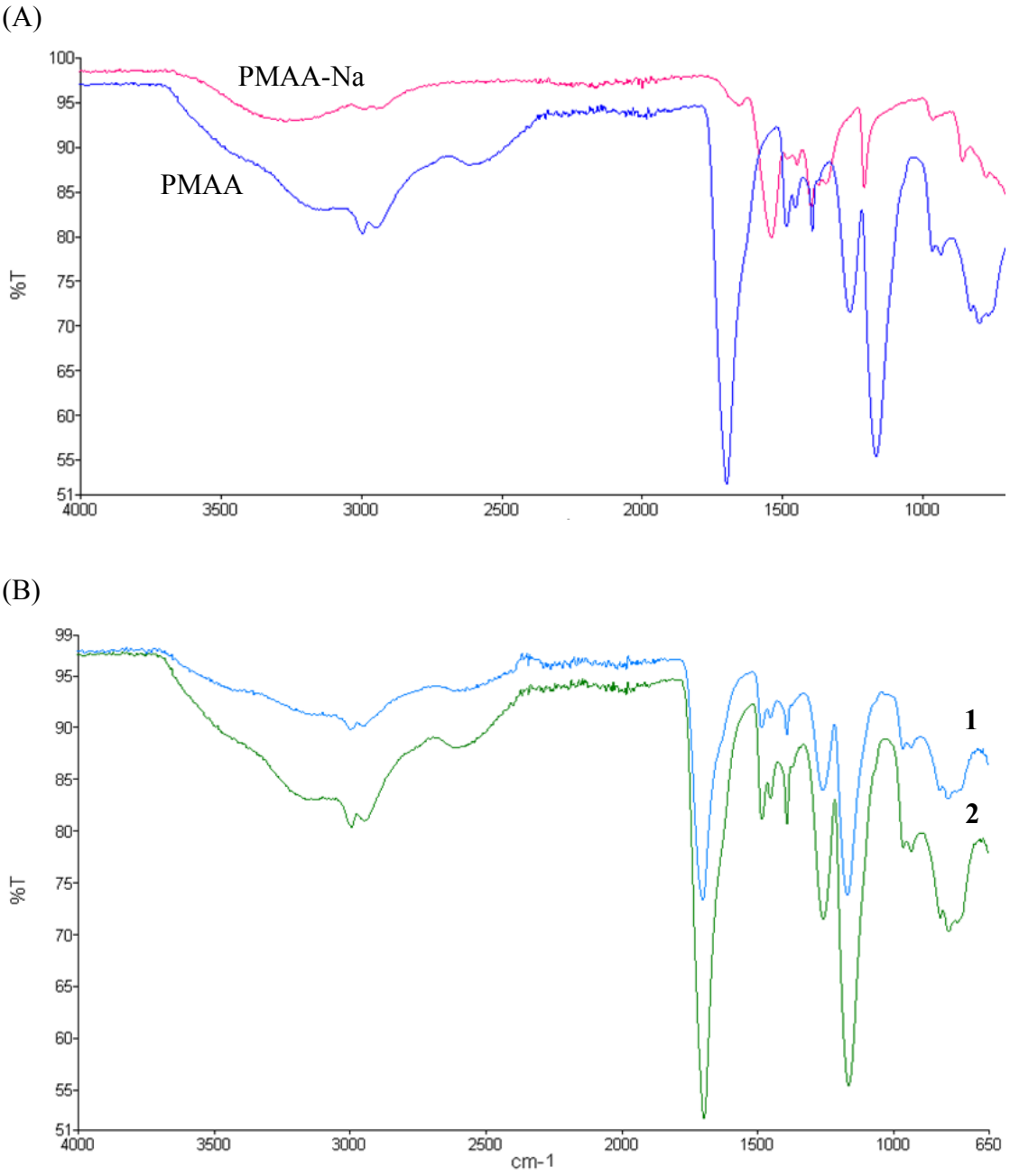


Figure S2. **(A)** Overlay of the FT-IR spectrum of PMAA and PMAA-Na. **(B)** Comparison of FT-IR spectra of PMAA protonated using salt precipitation method (1) and dialysis method (2).

Figure S3

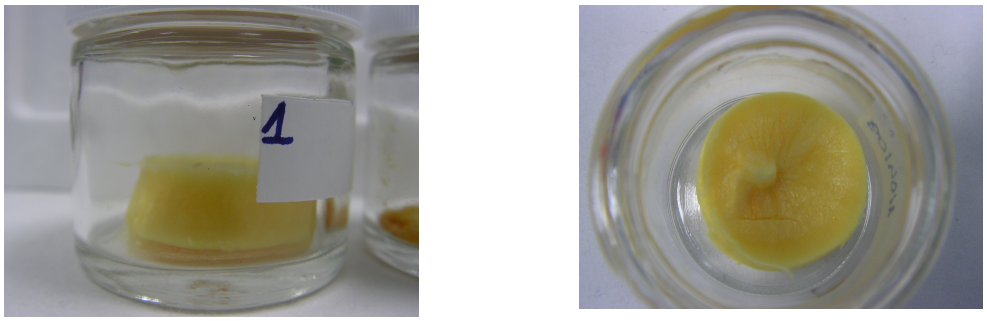
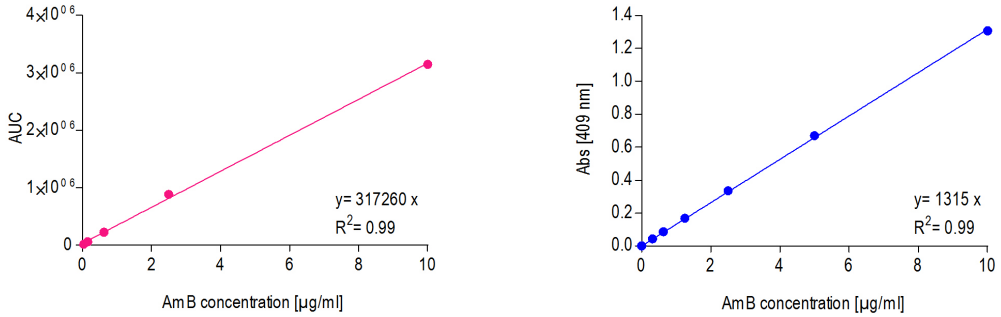


Figure S3. Photographic images of freeze-dried AmB-PMAA complex.

Figure S4



A B

Figure S4. AmB Calibration curves. **(A)** AmB in 50% methanol measured by UV-Vis and **(B)** RP-HPLC where elution was conducted with a 1 mL/min gradient starting from 30% acetinitrile in 25 mM EDTA to 100% acetonitrile (20 min) using a SUPERCOSIL™ LC-18-DB column. Both detected at 409 nm and N= 3.

Figure S5

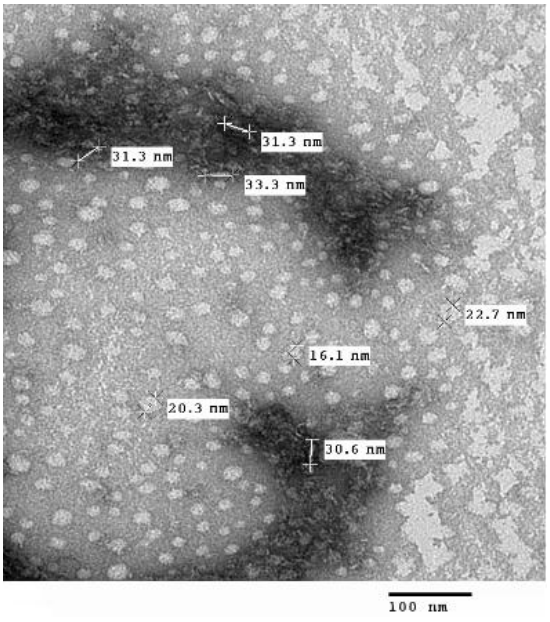


Figure S5. Image of the AmB-PMAA complex **3** by transmission electron microscopy (Direct Mag 93000). The AmB-PMAA complex contained 35 wt% AmB and was frabricated from PMAA with a molecular weight of 31.1 kDa.

Supplementary Tables

Table S1

Batch	PVP:PMAA (mol:mol)	AmB in complex [Wt%]	Amount PMAA used (mg)	Amount PVP used (mg)	Amount AmB used (mg)
1	0	2	0	12.5	12.5
2	0.5	19	20	26	20
3	1	35	20	0	20

Table S1. The AmB content in a complex prepared when using PVP (58 kDa) as the only polymer for complexation (Batch 1) was very low. Increased AmB loading was observed when the relative amount of PMAA (31.1 kDa) was also increased.

Table S2

AmB (mg)	PMAA (mg)	AmB content-UV (wt%)	AmB loading-HPLC (wt%)	AmB complexation efficiency (%)	Yield [%]
52	70	25	23	50	81
112	70	36	34	40	64
150	70	41	46	44	70
131	100	36	--	52	77
252	200	33	37	46	74

Table S2. AmB content, complexation efficiency and complex preparation yields for selected samples prepared using different ratios of AmB to PMAA at different scales of complexation.

Table S3

PMAA MWt (kDa)	AmB content (wt%)
7.5	35 ± 2
31.1	33 ± 5
100	28 ± 3
7.5*	30
31.1*	32 ± 3
65.5*	31 ± 1
100*	32

Table S3. The effect of the MW of PMAA used to prepare a complex and the AmB content in the final complex. All samples, prepared in triplicates, were prepared with a targeted AmB content of 30 wt% using the same weight ratios of AmB to PMAA. *PVP present.