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Polydopamine-on-Liposome: Stable nanoformulations, Uniform Coatings and Superior Antifouling Performance

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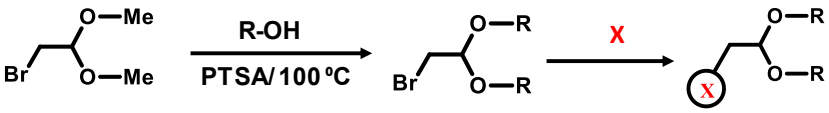
1. Synthesis of Cationic amphiphiles:

Cationic amphiphiles **L1**, **L2**, **L3** and **L4** were synthesised using a simple two-step synthesis protocol. In the first step, transacetalation of Bromoacetaldehyde dimethyl acetal was undertaken to attach the alkyl tails, and in the second step quaternisation reaction was carried out **(Figure S1).**

Step 1: (Transacetalation of Bromoacetaldehyde dimethyl acetal): p-Toluene sulfonic acid (PTSA) (52 mg, 0.1 eq., 0.296 mmol) was taken in a RB (100 mL) and mixed with acetone (25 mL) followed by addition of small amount of anhydrous sodium sulphate and kept on stirring for 20 minutes. Then filtrate was dried over anhydrous Na_2SO_4 and solvent was removed under low pressure to get activated PTSA catalyst. The activated PTSA catalyst was then dissolved in THF (5.0 mL) and Bromoacetaldehyde dimethyl acetal (350 µL, 1 eq., 2.96 mmol) was added to it with stirring. This was followed by addition of 1-dodecanol (3.36 mL, 4 eq, 14.9 mmol) to reaction mixture drop wise at RT. The reaction mixture was refluxed at 120 ºC for 18 hr. Reaction progress was monitored by TLC using hexane and ethyl acetate as eluents and developing the TLC in yellow dip. After completion of reaction solvent was evaporated on rotary evaporator to yield the crude product that was purified by solvent extraction, it was then washed with saturated sodium bicarbonate solution to neutralize the PTSA. Then organic layer was dried over anhydrous sodium sulfate and solvent was evaporated. The crude product was further purified by column chromatography top spot was collected (stationary phase was silica 100-200 mesh, and eluent phase was Hexane and ethyl acetate). Obtained (65%) as transparent liquid. ^{1}H NMR ($CDCl_3$) was recorded to characterise the product. Using similar protocol Bromoacetaldehyde di-cetyl acetal was also prepared.

Step 2: (Quaternization reaction): Pyridine or DMAP (2 mmol dissolved in 2 mL THF) were taken in a seal tube followed by drop wise addition of bromoacetaldehyde dilauryl acetal (1.3 eq, 2.6 mmols) with stirring at RT. Then reaction mixture was refluxed at 90 ºC in oil bath for

12 hr and the formation of desired product was monitored by TLC chloroform:methanol (90:10 v/v). The product was precipitated in cold diethyl ether to obtain the amphiphiles in sufficient purity. All the amphiphiles were characterized by ^{1}H-NMR and mass spectrometry (**Figure S10-S17**).



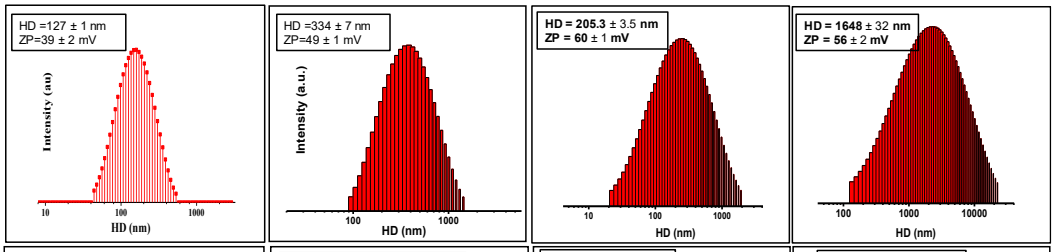
Name of the lipid	**L1**	**L2**	**L3**	**L4**
Head Group (X)	Pyridinium	N,N-dimethylamino pyridinium	Pyridinium	4,4′-bipyridinium
Tail (-R)	$-C_{12}H_{25}$	$-C_{12}H_{25}$	$-C_{16}H_{33}$	$-C_{12}H_{25}$

Figure S1. Synthesis scheme of cationic amphiphiles **L1**, **L2**, **L3** and **L4** and the molecular features of each amphiphile.

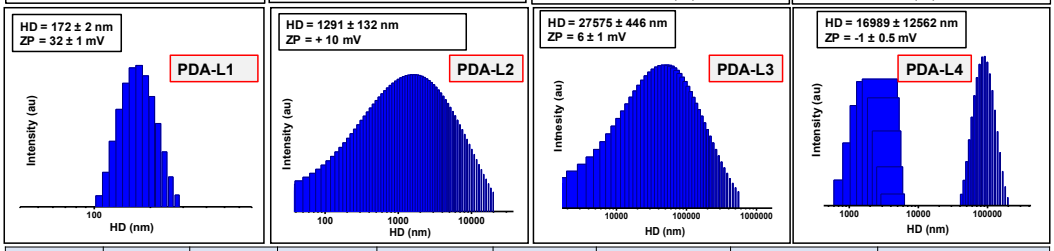
2. Hydrodynamic diameter and Zeta Potential of the amphiphiles and the PDA-Amphiphile formulations

The average hydrodynamic diameter (HD) and zeta potential (ZP) values for all the four cationic amphiphiles were determined by dynamic light scattering (DLS) using a Delsa Nano (Beckman Coulter) instrument with the CONTIN algorithm. For recording zeta potential, the amphiphiles were further mixed with NaCl (20 mM) and then ZP was recorded. Corresponding PDA-Amphiphile formulations were prepared and DLS experiments were carried out to obtain HD and ZP values. Concentration of amphiphiles were fixed to 2 mg/mL and the DA was 30 mg/mL. HD and ZP data is shown in **Figure S2**.

A.



B.



C.

	L1	L2	L3	L4	PDA-L1	PDA-L2	PDA-L3*	PDA-L4*
HD (nm)	127 ± 1	334 ± 7	205 ± 3	1648 ± 32	172 ± 2	1291 ± 132	27575 ± 446	16989 ± 12562
ZP (mV)	39 ± 2	49 ± 1	60 ± 1	56 ± 2	32 ± 1	10 ± 3	6 ± 1	-1 ± 0.5

Figure S2. Hydrodynamic diameter (HD) and zeta potential (ZP) of **(A)** amphiphile **L1, L2, L3**, and **L4** (2 mg/mL solution in water) and **(B)** of **PDA-L1**, **PDA-L2**, **PDA-L3**, and **PDA-L4** samples; **(C)** Summary of the HD and ZP values for the different samples. (*These HD values were outside the reliable estimate range of the instrument).

3. Investigation of the local pH with phenol red

A 1 mM stock solution of Phenol red was made in distilled water. 10 µL of this yellow colored solution was added to liposomal solutions of **L2**, **L3** and **L4** containing 2.0 mg/mL amphiphile. The amphiphile formulations immediately acquired purple coloration. The samples were allowed to equilibrate for 10 min and their UV-Vis spectra were recorded to assess the local pH (**Figure S3**).

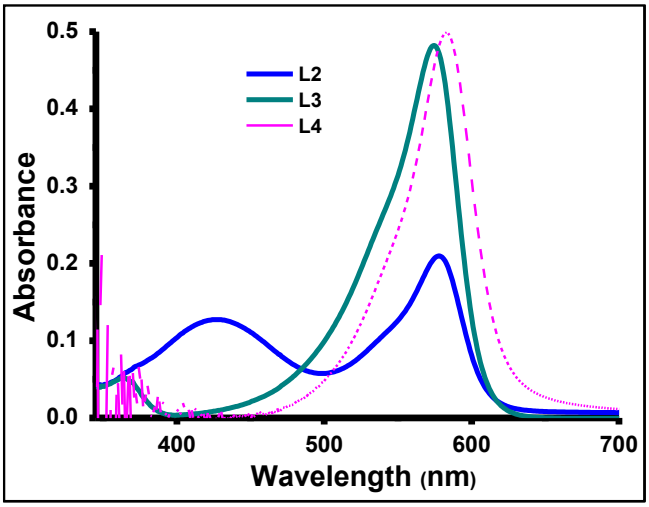


Figure S3. UV-vis spectrum of 10 µM phenol red in aqueous solutions of amphiphiles **L2**, **L3** and **L4** (2 mg/mL).

4. Preparation and characterisation of base-polymerized polydopamine (BP-PDA)

Solid dopamine hydrochloride (DA, 10-30 mg) was added to 1 mL water pre-adjusted to pH = 8.5 using sodium hydroxide solution. The colour of aqueous medium started turning brown almost immediately due to the polymerisation of DA. After 6 hr, a turbid suspension was obtained that finally turned black with visual precipitates in the suspension. Dialysis against deionized water with 3 kDa cellulose nitrate membrane was performed to remove the salts. DLS and microscopies were done on this sample (**Figure S4**) shown below:

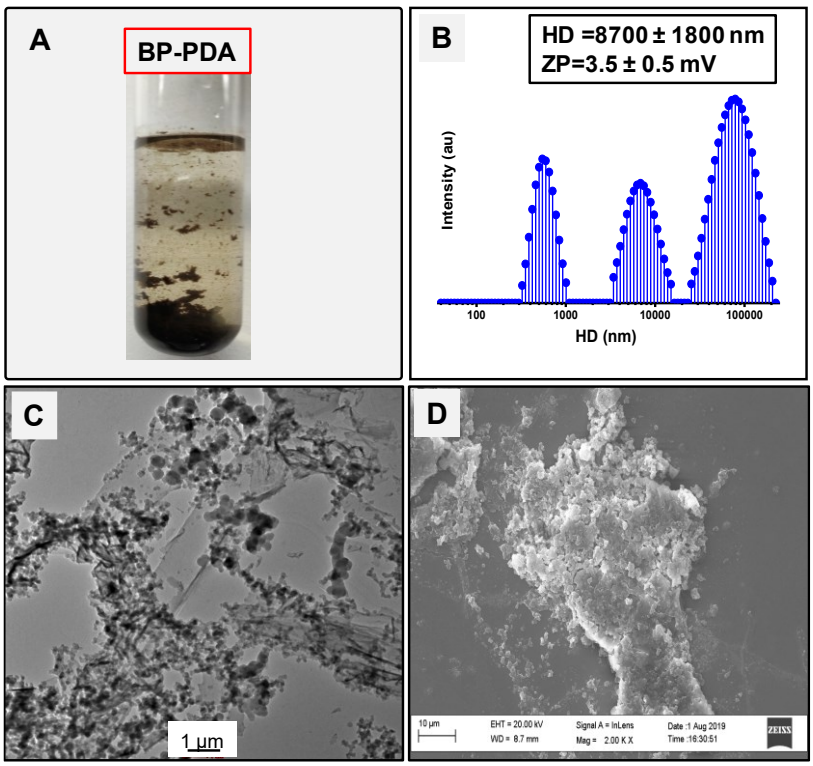


Figure S4. (A) Digital photograph, **(B)** hydrodynamic diameter from DLS and **(C)** TEM image of **BP-PDA** sample. **(D)** SEM image of coating formed by dipping glass coverslip in **BP-PDA**.

5. Stability of PDA-L1 coatings in simulated body fluids

To assess the long term stability of **PDA-L1**, simulated body fluid (SBF) was prepared as per reference: “T. Kokubo, H. Kushitani, S. Sakka, T. Kitsugi and T. Yamamuro, *J. Biomed. Mater. Res.,* 1990, **24**, 721-734.” Then the glass coverslips coated with **PDA-L1** and **BP-PDA** as control were dipped in SBF and SEM images were taken at different point of time in order to check the stability of coatings created by **PDA-L1** and **BP-PDA**. Both coatings were found to be stable in SBF.

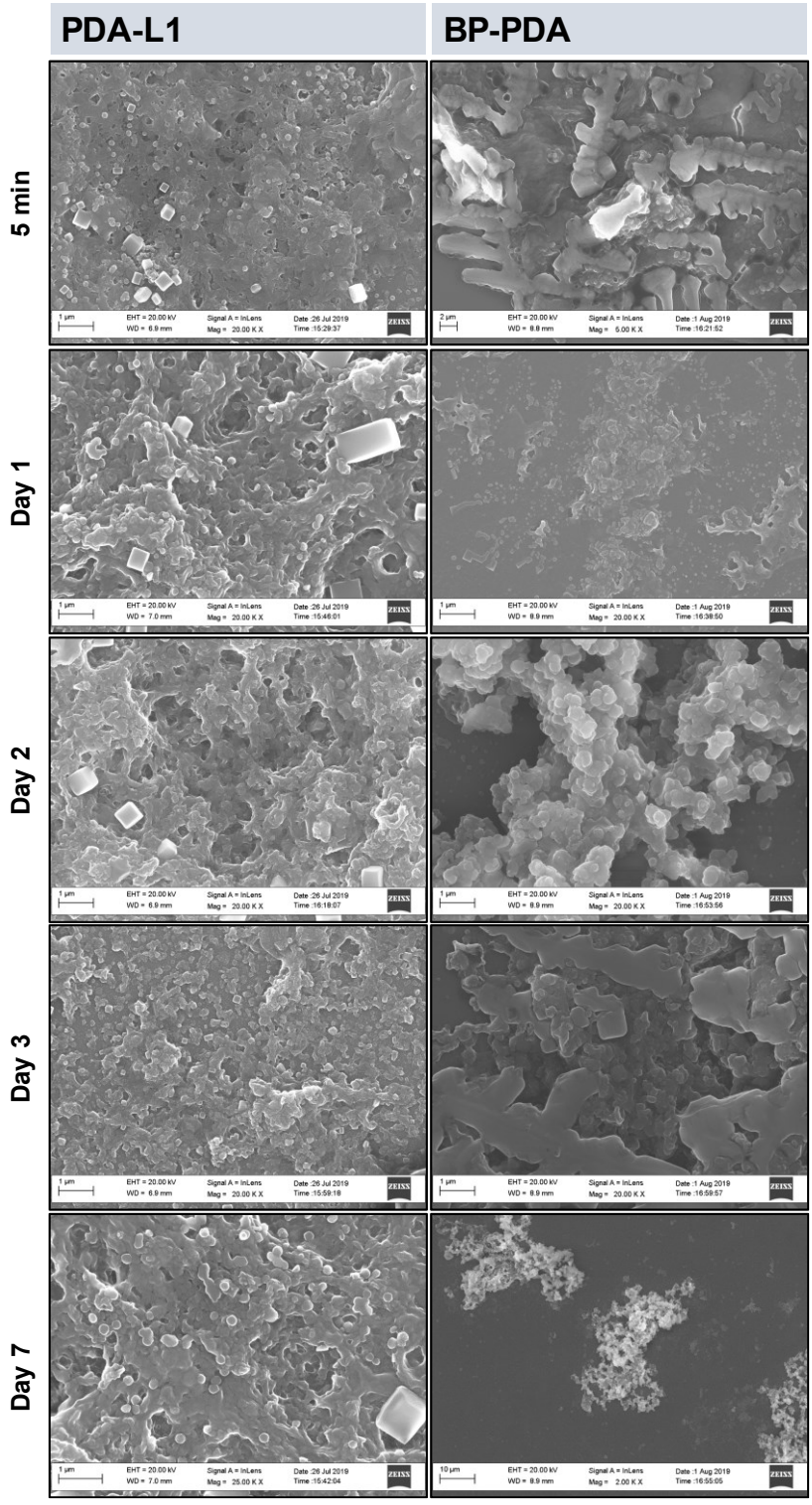


Figure S5. Stability of **PDA-L1** and **BP-PDA** coatings in simulated body fluid.

6. Stability of PDA-L1 coatings at different pH

PDA-L1 coated latex catheter pieces were immersed in phosphate buffer of pH 5.6, 7.4 or 8.0. Then the morphology of coatings were visualised under SEM at different time points (Day 0, 1, 2, 3) (**Figure S6).**

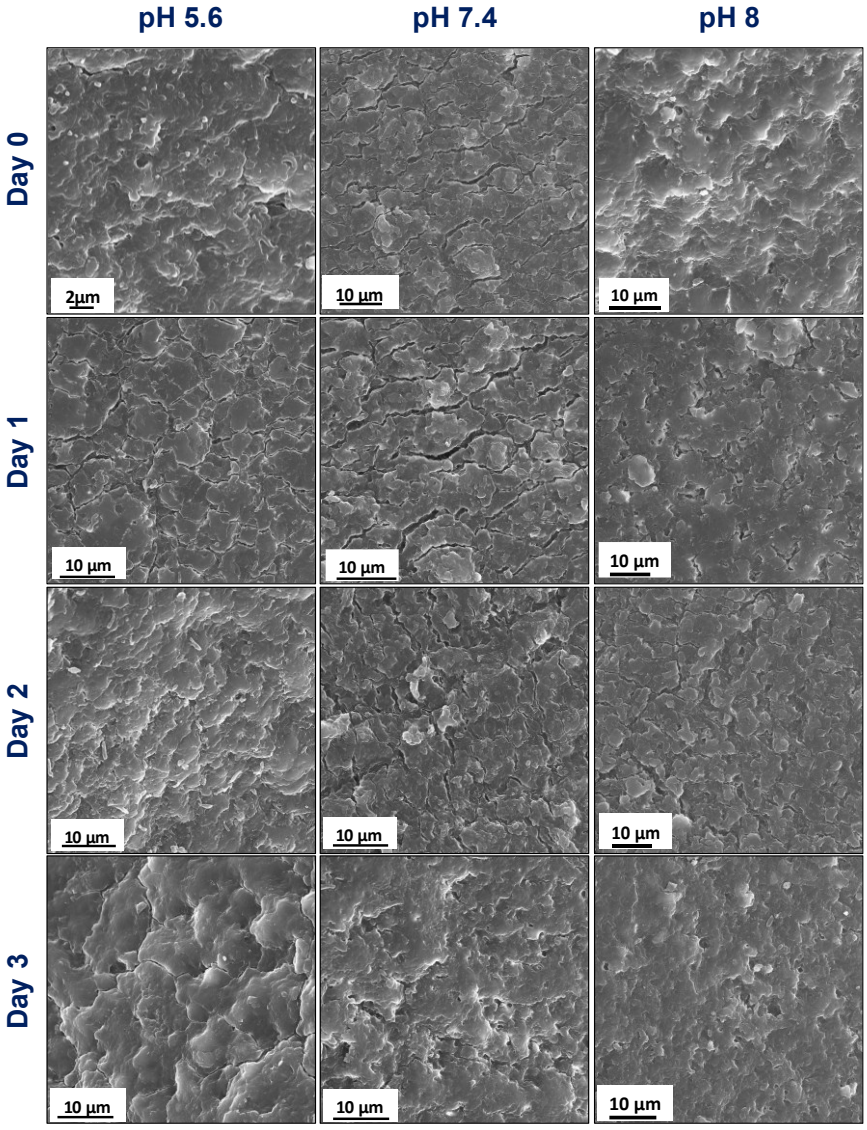


Figure S6. Morphology of **PDA-L1** coatings on catheter upon immersion in phosphate buffer of different pH. SEM images taken after every 24 hr for three days.

7. Stability of PDA-L1 coatings against the enzymes

1 wt% pepsin solution was prepared in 50 mM HCl/KCl buffer of pH 2 and 1 wt% pancreatin solution was prepared in 50 mM phosphate buffer of pH 7.4. Then **PDA-L1** coated latex catheter pieces were immersed in both the enzyme solution separately. It was then visualised under SEM (**Figure S7).**

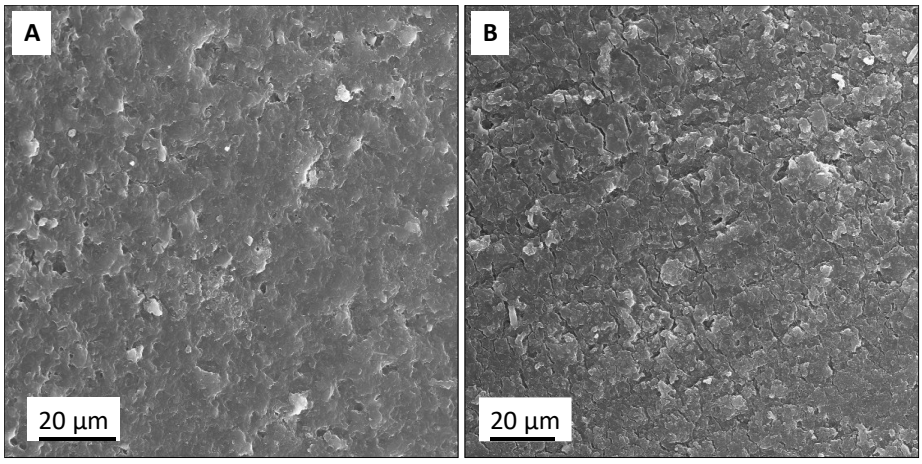


Figure S7. Morphology of **PDA-L1** coating after immersing for 12 hr in **(A)** 1 wt% pepsin in HCl/KCl buffer of pH 2; **(B)** 1 wt% pancreatin in phosphate buffer of pH 7.4.

8. Adherence of FITC labelled Bovine serum albumin (BSA) on PDA-L1 coatings

Bovine serum albumin (BSA) was labelled with FITC using the protocol as per reference "Nadia Barbero, Claudia Barolo, Guido Viscardi, *World J. Chem. Educ.* 2016, **4**, 80-85. " Then non-coated, **PDA-L1** coated and **BP-PDA** coated glass slides were dipped in BSA-FITC solution and left immersed for 1 hr. The coverslips were then taken out and washed 3 times with distilled water and then fluorescence images were taken using a fluorescence microscope.

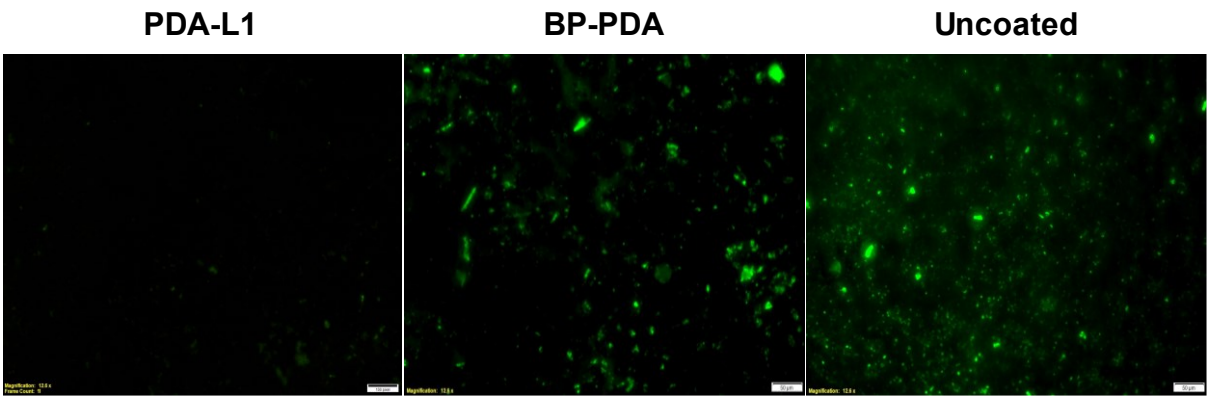


Figure S8. Fluorescence microscopy images on dipping the glass coverslips coated with **BP-PDA** and **PDA-L1** in FITC-BSA solution for 1 hr, scale bar 50 μm.

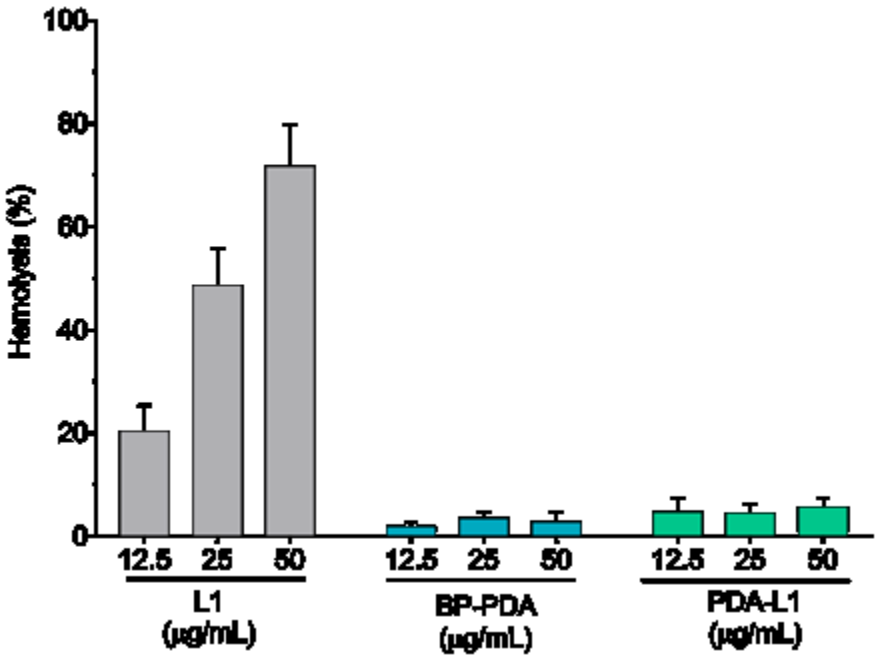


Figure S9. Haemolytic activities of **L1**, **BP-PDA** and **PDA-L1** formulations at different concentrations.

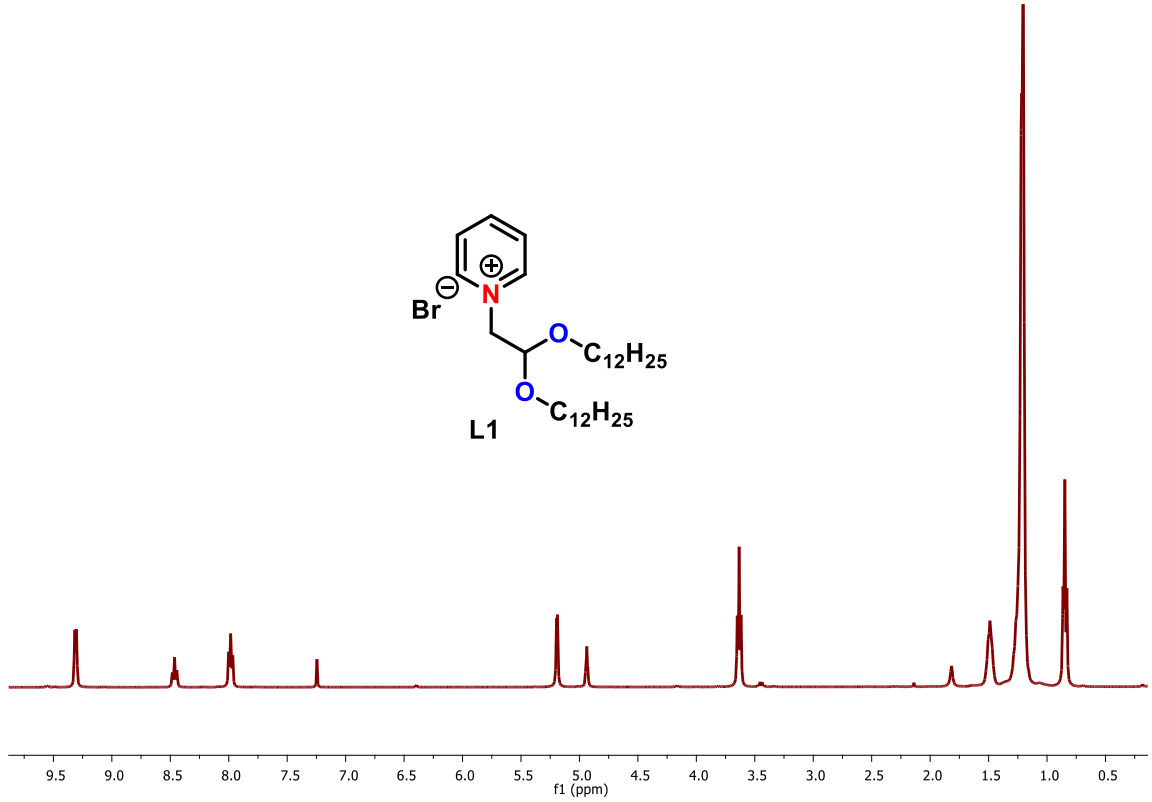


Figure S10. ^{1}H-NMR of Amphiphile **L1** at 400 MHz in $CDCl_3$

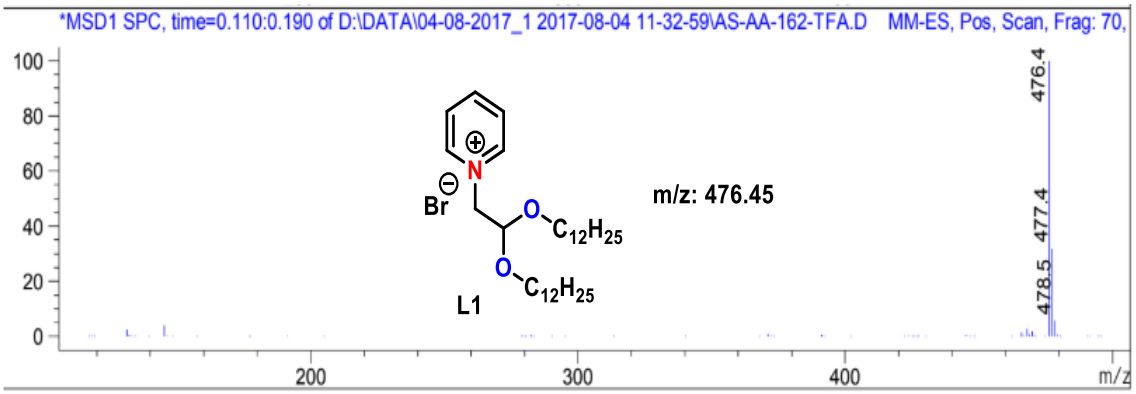


Figure S11. LC-LRMS characterisation of amphiphile **L1**

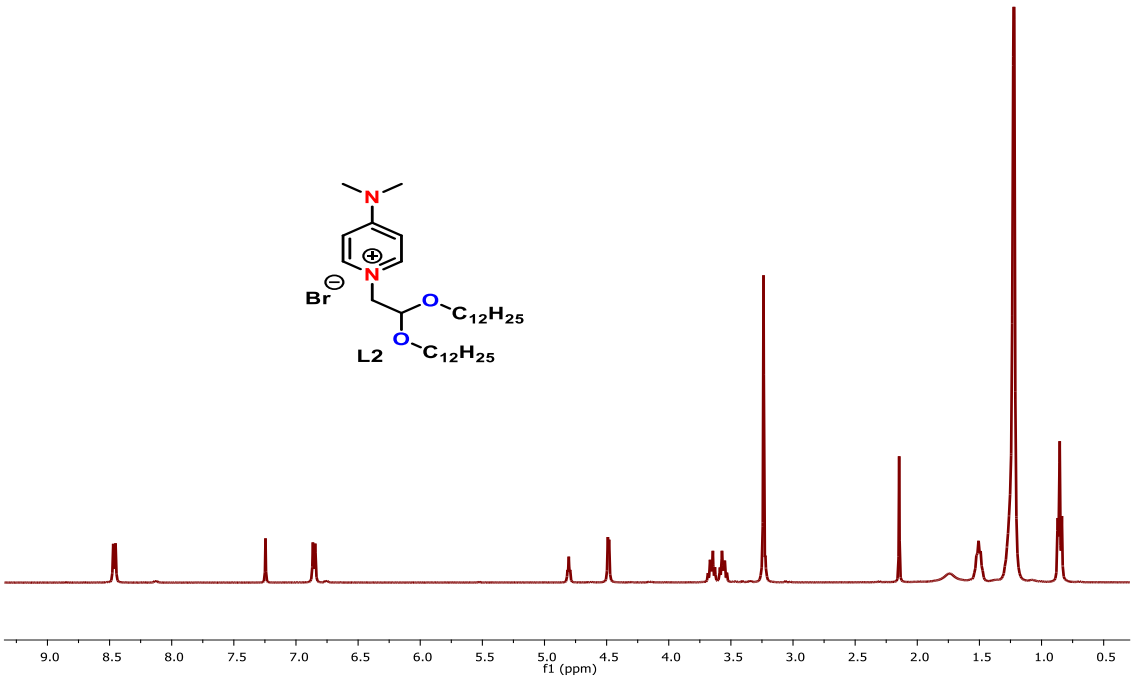


Figure S12. ^{1}H-NMR of amphiphile **L2** recorded at 400 MHz in $CDCl_3$

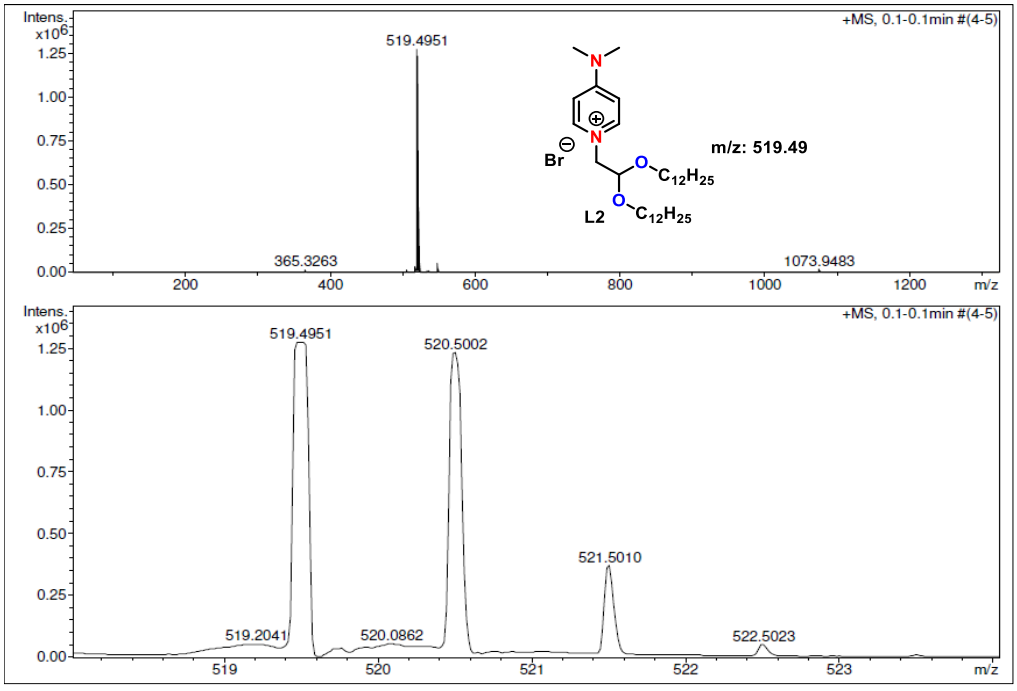


Figure S13. ESI-HRMS characterisation of amphiphile **L2**

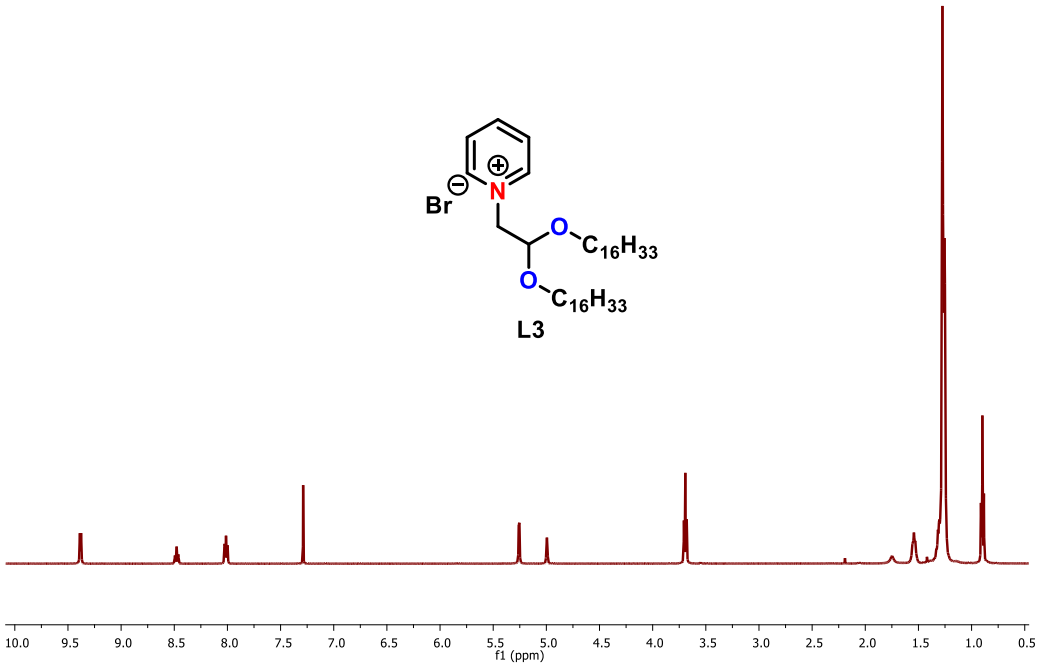


Figure S14. ^{1}H-NMR of amphiphile **L3** recorded at 400 MHz in $CDCl_3$

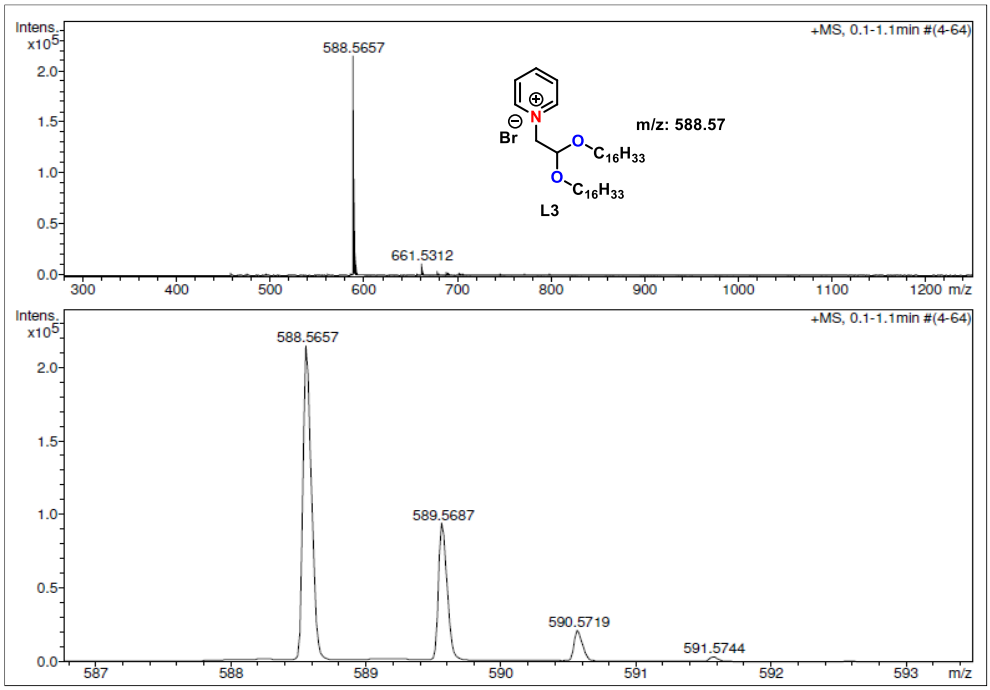


Figure S15. ESI-HRMS characterisation of amphiphile **L3**

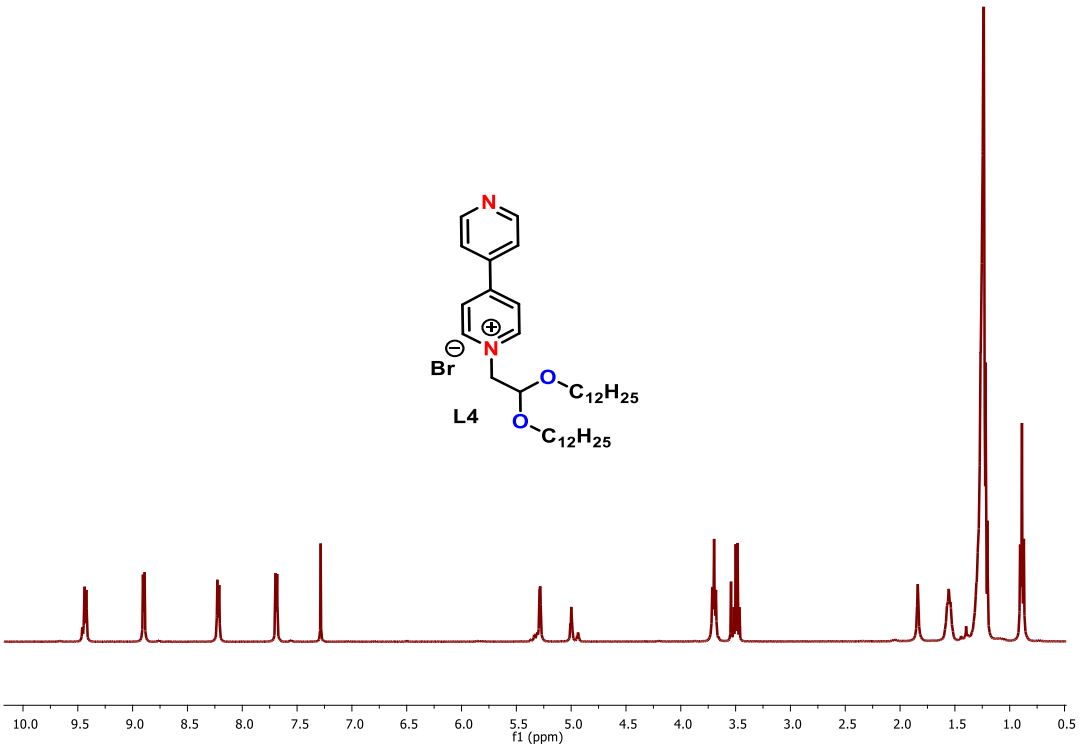


Figure S16. ^{1}H-NMR of amphiphile **L4** recorded at 400 MHz in $CDCl_3$

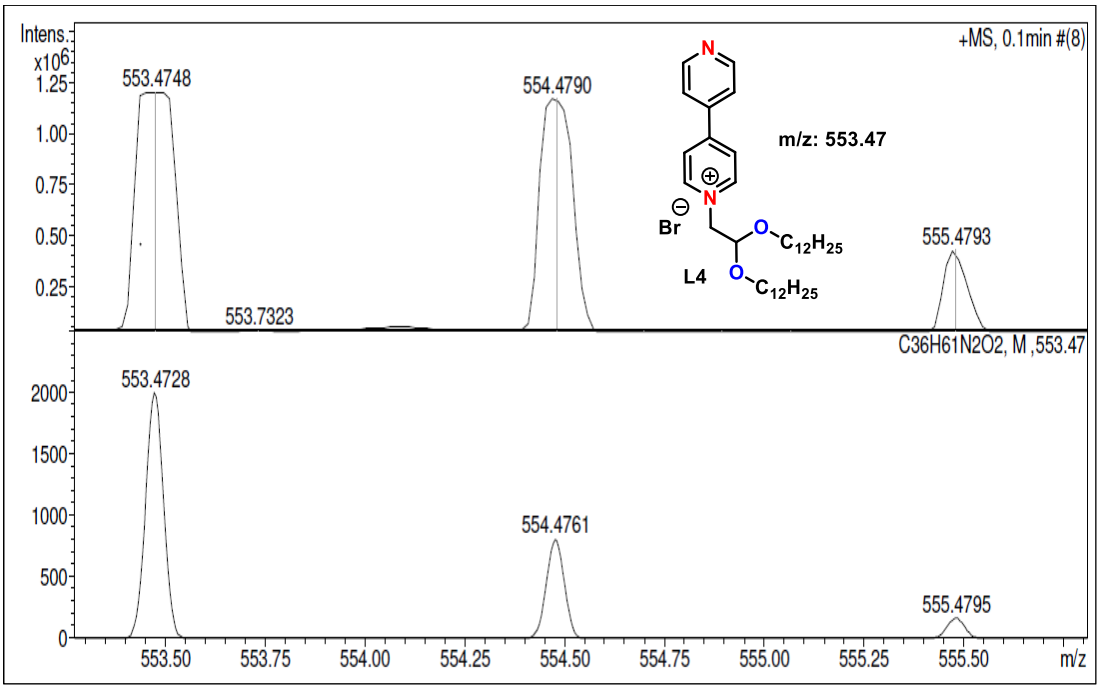


Figure S17. ESI-HRMS characterisation of amphiphile **L4**