Electronic Supplementary Information for:

Water-dispersible and biodegradable polymer micelles with good antibacterial efficacy

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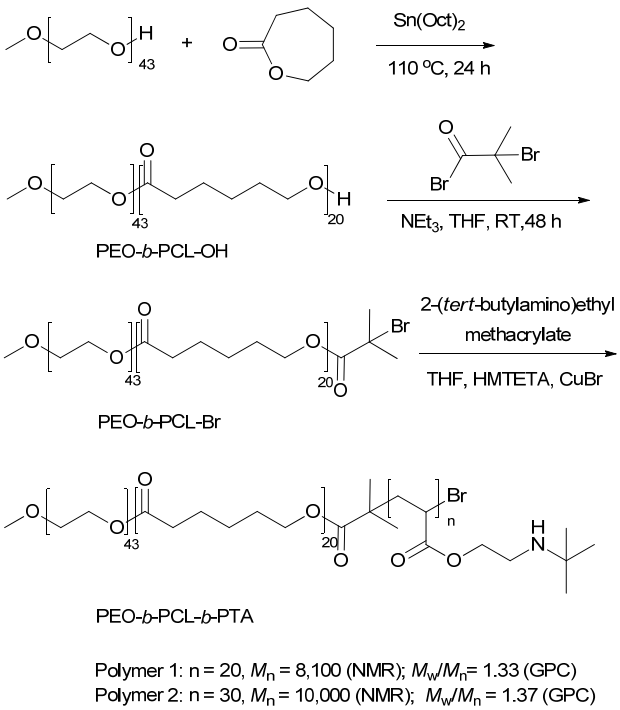
Scheme S1. Synthesis of PEO-*b*-PCL-*b*-PTA triblock copolymers.



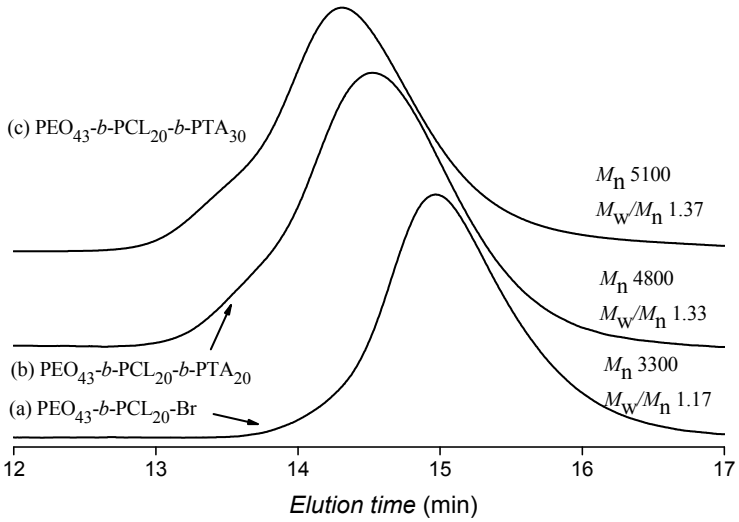


Fig. S1. GPC traces of block copolymers in DMF. The corresponding molecular weights by ^{1}H NMR are 4300, 8100 and 10000 from a to c, respectively. The discrepancy in the GPC and NMR molecular weight is due to their different principles for determining molecular weights.

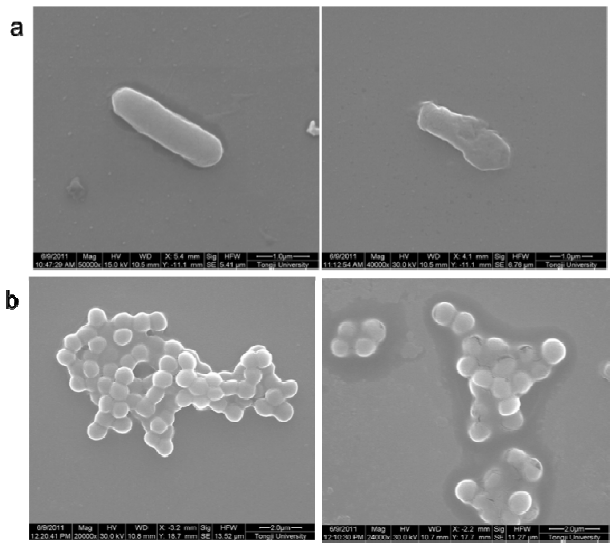


Fig. S2 SEM images of microbes in the absence and presence of polymer 2 micelles: (a) E. coli or (b) S. aureus is before (left) and after (right) incubation with polymer 2 micelles for 8 h at lethal doses.

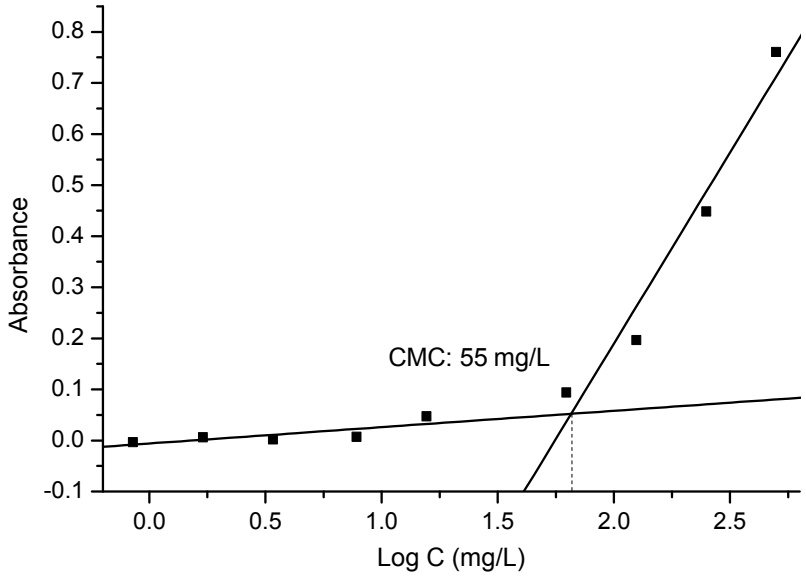


Fig. S3 UV absorbance of pyrene at 242 nm as a function of the concentration of PEO_{43}-*b*-PCL_{20}-*b*-PTA_{30} (polymer 2) in water. This experiment confirmed the CMC of polymer 2 is 55 mg/L (5.5 μM).

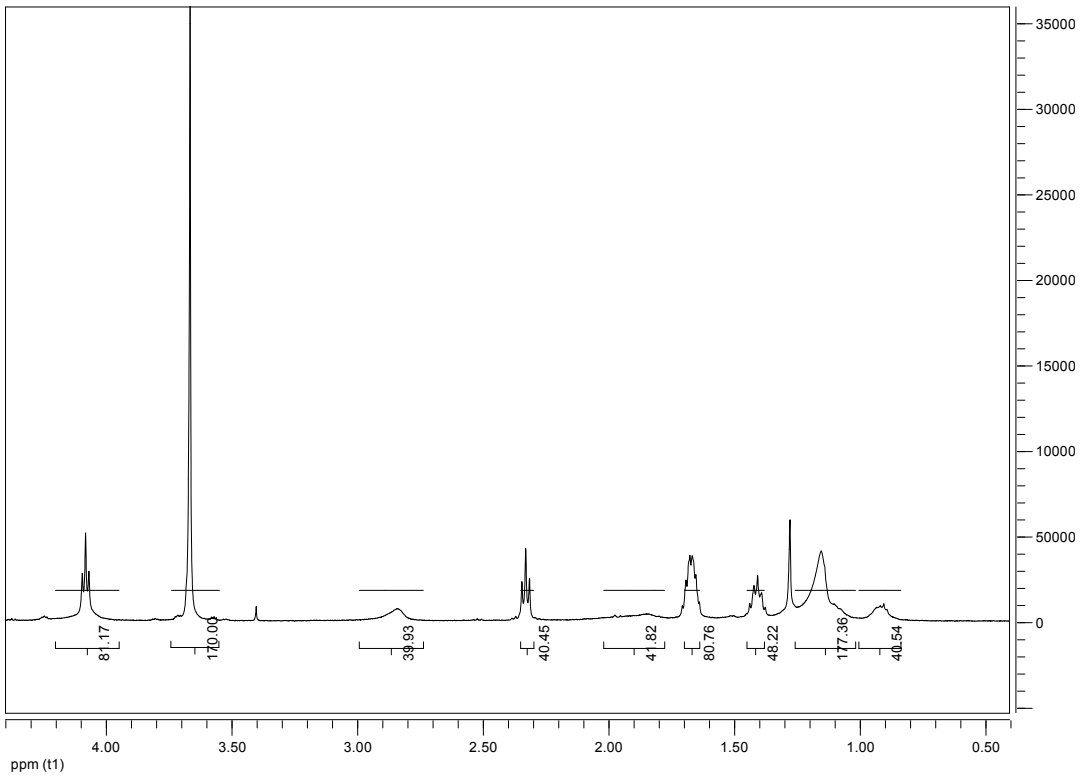


Fig. S4 ^{1}H NMR spectrum of PEO_{43}-*b*-PCL_{20}-*b*-PTA_{20} (polymer 1) triblock copolymer in $CDCl_3$.

For assignment see Fig. 1 in the main text.

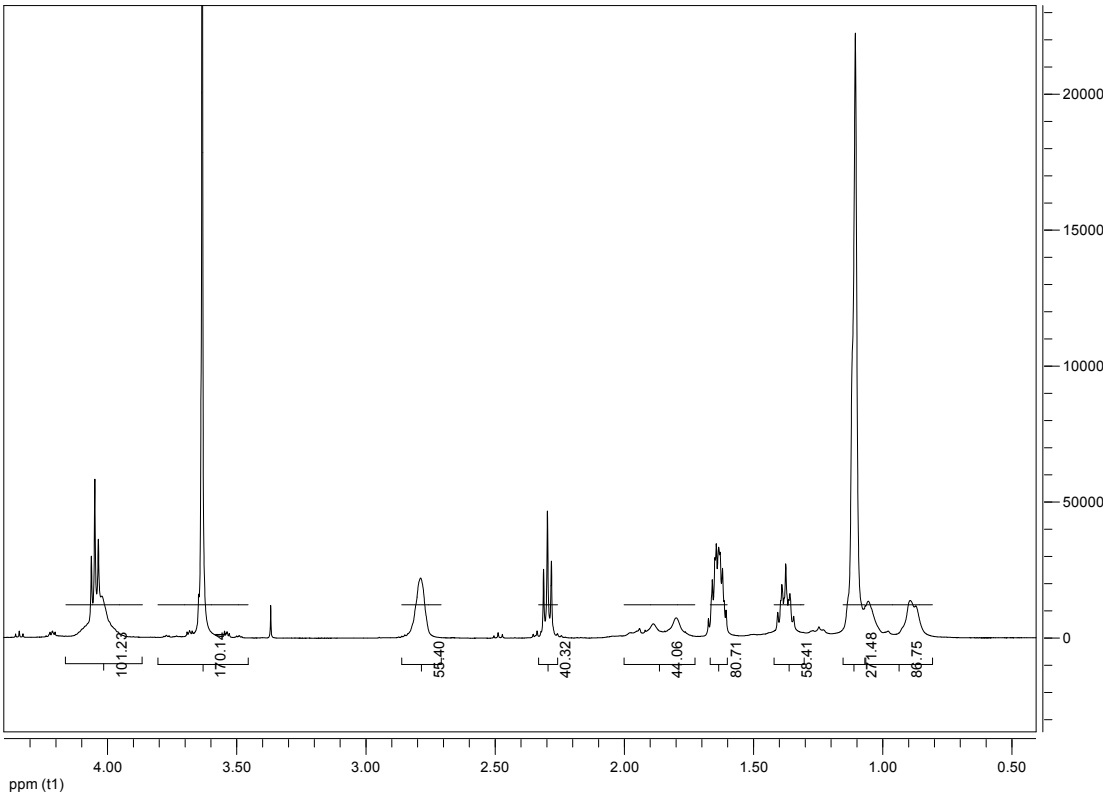


Fig. S5 ^{1}H NMR spectrum of PEO_{43}-*b*-PCL_{20}-*b*-PTA_{30} (polymer 2) triblock copolymer in $CDCl_3$.

For assignment see Fig. 1 in the main text.

Determination of the composition of PEO_{43}-*b*-PCL_x-*b*-PTA_y triblock copolymers by ^1H NMR

According to the above ^1H NMR spectra (see Fig. 1 for assigment), the degrees of polymerization of both copolymers were calculated according to the following procedures.

Table S1. Summary of the areas of different peaks and the degrees of polymerization of PEO_{43}-*b*-PCL_x-*b*-PTA_y triblock copolymers

Spectrum	Polymer	a_b	a_d	a_l	a_{i+k}	a_m	x	y
Fig. S4	1	170	40.5	39.9	81.2	177.4	20	20
Fig. S5	2	170	40.3	55.4	101.2	271.4	20	30

In **Table S1**, a_b, a_d, a_l and a_{i+k} are the areas of the peaks b (PEO, 170 H), d (PCL, 2x H), l (PTA, 2y H) and i+k (PCL and PTA, 2x + 2y), m (PTA, 9y H) in **Fig. S4** and **Fig. S5**.

We set that the integration area of peak b is 170, which corresponds to the amount of H in PEO_{43} ($43 \times 4 - 2 = 170$). The areas of other peaks are listed in Table S1.

Fig. S4 (Polymer 1): according to separated peaks b (PEO) and d (PCL), l (PTA), m (PTA), and overlapped i+k (PCL and PTA):

Block length of PCL (comparing peak b with separated peak d):

$$x = \frac{40.5}{170} \times \frac{43 \times 4 - 2}{2} = 20.25 \approx 20$$

Block length of PTA (comparing peak b with separated peak l):

$$y = \frac{39.9}{170} \times \frac{43 \times 4 - 2}{2} = 19.95 \approx 20$$

Block length of PTA (comparing peak b with separated peak m):

$$y = \frac{177.4}{170} \times \frac{43 \times 4 - 2}{9} = 19.71 \approx 20$$

Block length of PTA (comparing peak b with overlapped peak i+k):

$$y = \frac{81.2 - 40.5}{170} \times \frac{43 \times 4 - 2}{2} = 20.35 \approx 20$$

Fig. S5 (Polymer 2): according to separated peaks b (PEO) and d (PCL), l (PTA), m (PTA), and overlapped i+k (PCL and PTA):

Block length of PCL (comparing peak b with separated peak d):

$$x = \frac{40.3}{170} \times \frac{43 \times 4 - 2}{2} = 20.15 \approx 20$$

Block length of PTA (comparing peak b with separated peak l):

$$y = \frac{55.4}{170} \times \frac{43 \times 4 - 2}{2} = 27.7 \approx 28$$

Block length of PTA (comparing peak b with separated peak m):

$$y = \frac{271.4}{170} \times \frac{43 \times 4 - 2}{9} = 30.16 \approx 30$$

Block length of PTA (comparing peak b with overlapped peak i+k):

$$y = \frac{101.2 - 40.3}{170} \times \frac{43 \times 4 - 2}{2} = 30.45 \approx 30$$

Experimental Section

Materials:

ε-Caprolactone (Aldrich) was dried azeotropically using anhydrous toluene to remove traces of water. Poly(ethylene oxide) (molecular weight is 1900) was purchased from Alfa Aesar, dried azeotropically using anhydrous toluene prior to use. 2-(*tert*-Butylaminoethyl) methacrylate (TA) was purchased from J & K, dried over CaH_2 overnight, and distilled under reduced pressure prior to use. Toluene was dried by reflux and distillation in the presence of sodium and benzophenone. Cu(I)Br was washed with acetic acid and ethanol alternatively for five times and then dried in vacuum. 1,1,4,7,10,10-Hexamethyltriethylenetetramine (HMTETA) were purchased from J & K and used as received. Stannous 2-ethylhexanoate ($SnOct_2$, approx. 95%), triethylamine, 2-bromoisobutyryl bromide, THF, $CHCl_2$, and n-hexane were purchased from Aladdin and used as

received.

The bacteria used in this work consisted of Gram-negative *E. coli* bacteria of DH52 strain and Gram-positive *S. aureus* bacteria of ATCC29253 strain, which were kindly donated by Institute for Advanced Materials and Nano Biomedicine, Tongji University. Liquid Luria–Bertani (LB) medium (final pH is 7.4) contains the following: 10.0 g tryptone, 5.0 g yeast extract and 10.0 g sodium chloride. Trypcasein Soytone Broth and Trypcasein Soy Agar were purchased from BD.

Characterization:

DMF GPC. GPC analysis was carried out with a Waters Breeze 1525 GPC analysis system with two PL mix-D column, using DMF with 0.5 M LiBr as eluents at a flow rate of 1.0 mL min^{-1} at 80 ^{o}C. PEO calibration kit (purchased from TOSOH) was used as the calibration standard.

^{1}H NMR spectra were recorded using a Bruker AV 400 MHz spectrometer at ambient temperature using $CDCl_3$ as solvent.

Transmission electron microscopy (TEM) images were obtained using a JEM-2100 electron microscope equipped with a Gatan 1K ×1K digital camera operating at an acceleration voltage of 200 kV. To prepare TEM samples, 10 μL of diluted polymer micelle solution was placed on a copper grid coated with thin carbon film and dried in air. The samples were stained by 1% phosphotungstic acid.

Scanning electron microscopy (SEM). SEM images were obtained using Quanta 200 FEG electron microscope operated at 15 kV. Samples for the SEM studies were prepared from the micellar solution by exposing the bacteria to the loaded micelles. The bacteria solution was then centrifuged and the supernatant was replaced by glutaraldehyde. After re-dispersion of the bacteria in the fixative and letting the sample stay in the fridge for two days, the bacteria sample was transferred onto silicon slice using micropipette, waiting for 4 hours for moisture volatiles.

Afterwards, the samples were dehydrated with acetone/water mixture solution at increasing concentrations of acetone of 30%, 50%, 70%, 80%, 90% and 100% (vol.%) for 3 times in every solution in 15 min. Then the samples were freeze-dried for 48 hours. Finally the samples were sprayed with nano-gold.

DLS studies were conducted using a Zetasizer Nano ZS90 instrument (Malvern Instruments) equipped with a multipurpose autotitrator (MPT-2) at a fixed scattering angle of 90°. The data were processed by cumulants analysis of the experimental correlation function. The particle diameters were calculated from the computed diffusion coefficients using the Stokes-Einstein equation. Each reported measurement was conducted for three runs.

Synthesis of PEO-*b*-PCL-OH: The diblock copolymer was synthesized according to a reported method.[1] Typically, a three-neck flask charged with a magnetic flea, PEO_{43}-OH (6.000 g, 3.158 mmol), ε-caprolactone (7.280 g, 63.20 mmol) and anhydrous toluene (60 g) was placed into an oil bath at 144 °C. Trace of water in the flask was removed by azeotropic distillation under Argon protection. About 30 g of toluene was distilled out. Then, the temperature was set at 110 °C. The oxygen was removed by bubbling with Agron for 30 min. Then $Sn(Oct)_2$ (200.0 μL, 2×10^{-4} mol) was added by micro pipette under nitrogen protection. After 24 h reaction, the flask was cooled down to room temperature. The product was purified by precipitation in hexane and dried under vacuum at 30 °C for 2 days. The final product is white powder(11.89 g).Yield: 90%.

Synthesis of PEO-*b*-PCL-Br: The synthesis was preformed according to a reported method.[1] Typically, PEO_{43}-*b*-PCL_{20}-OH diblock copolymer (8.000 g, 1.764 mmol) was dissolved in 40 mL of anhydrous THF. Then, it was placed into an ice-water bath. After adding TEA (2.13 mL, 15.30 mmol), the 2-bromoisobutyryl bromide (0.95 mL, 15.30 mmol) was added at a rate of 1 drop/10 s. Some insoluble salts produced instantly after adding 2-bromoisobutyryl bromide. After 24 h, the insoluble salts were removed by filtration. THF was removed using a rotary evaporator. Then, CH_2Cl_2 was added and washed with water and the organic phase was collected. The activated

carbon black and $MgSO_4$ was added into a CH_2Cl_2 solution, stirred for half a day and filtered. The CH_2Cl_2 solution was then concentrated. Hexane was added to precipitate the copolymers. The products were dried under vacuum at 30 ^oC for 2 days. The final product is white powder (5.235 g).Yield: 61%.

Synthesis of PEO-*b*-PCL-*b*-PTA triblock copolymers by ATRP: In a typical ATRP procedure, a flask with a magnetic flea and a rubber septum was charged with PEO-*b*-PCL-Br macroinitiator (2.000 g, 0.4499 mmol) and toluene (3.0 mL). After full dissolution of PEO-*b*-PCL-Br in toluene, TA monomer (2.583 g, 5.720 mmol) and HMTETA ligand (126.2 μL, 0.4499 mmol) were added. This solution was deoxygenated by bubbling with Ar for 30 min before adding CuBr (64.53 mg, 0.4499 mmol). The molar ratio of [TA]: [PEO-*b*-PCL-Br]: [CuBr]: [HMTETA] was 31: 1: 1: 1. The reaction was carried out under Argon protection at 70 ^oC. After 20 h, the reaction solution was diluted with THF and eluted through Al_2O_3 column to remove catalyst. The THF was removed using a rotary evaporator, then add CH_2Cl_2 to dissolve the triblock polymer. Then, hexane was added to precipitate the block copolymers. The product was dried under vacuum at 30 ^oC for 2 days. The final product is white powder (3.126 g).Yield: 68%.

Self-assembly of PEO-*b*-PCL-*b*-PTA triblock copolymer to micelles: The following protocol was adopted to self-assemble the triblock copolymer into micelles. PEO-*b*-PCL-*b*-PTA copolymer (50.00 mg) was dissolved into 5.0 mL THF. Then deionized water (10 mL) was added at a rate of 1 drop/10 s under vigorous stirring. Then the solution was dialyzed against water with a dialysis tube (8,000–14,000 molecular weight cut-off) at 25 °C. The solution pH was 7.8 (without any pH adjustment). Aqueous HCl solution was used to adjust the pH of the solution to 7.4.

Antibacterial test: Briefly, polymer micelle solution (30 mg/mL) was placed into each well of conical flask with 10 mL culture solution to certain concentrations. The microorganism solution (20 μL) which had an optical density reading of ~0.7-0.8 at 600 nm wavelength was added to each well. The optical density readings of microorganism solutions were measured as a function of time. The MIC was taken at the concentration where no growth was observed with visible spectrophotometer in the growing phase of the microorganisms. Broth containing cells alone was

used as control and the tests were repeated at least three times.

Colony assay. The counts of live microbes in the treated microbial samples were estimated by a colony formation assay. After 2, 4, 6, 8 hours of incubation with polymer micelles at various concentrations, 1.00 mL microbial solution was transferred with pipettor and diluted with an appropriate dilution factor, and then paced on an agar plate (LB Agar for *E. coli*, TSA for *S. aureus*). The plate was then incubated overnight at 37 ^{o}C. The number of colony-forming units (CFU) was counted and expressed as CFU/mL. Percentage of the counts of live microbe in the treated sample as compared to the counts in the control sample without any treatment was estimated as a function of polymer/antimicrobial concentration. The MBC was taken at the concentration where 99.9% microbes are killed.

Critical micelle concentration (CMC). The CMC of the PEO_{43}-*b*-PCL_{20}-*b*-PTA_{30} triblock copolymer was determined by UV-vis spectra using pyrene as a hydrophobic probe. 2.0 mg pyrene was dissolved in 10 mL acetone and 10 μL of solution was added into each cuvette. The acetone was allowed to evaporate. Then 4.0 mL of aqueous PEO_{43}-*b*-PCL_{20}-*b*-PTA_{30} solution ranging from 0.85 mg/L to 500 mg/L were added into the pyrene-containing cuvette separately, resulting in a theoretically maximum pyrene concentration of 2.0 μM in water. Upon sonication for 10 min, the solutions were kept at room temperature and equilibrated for 24 h before UV measurements. The spectra were recorded in the 200–400 nm wavelength range. The obvious absorption peaks for pyrene were at 242, 272, 320 and 336 nm and the CMC was estimated by extrapolating the absorbance at 242 nm at various polymer concentrations. See Fig. S3 for the CMC value.

Do the possible residual Sn compounds affect the antibacterial activity of polymer micelles?

Recipe of synthesis of PEO-*b*-PCL-OH: PEO_{43}-OH (6.000 g), ε-caprolactone (7.280 g), $Sn(Oct)_2$ (2×10^{-4} mol).

Assuming no Sn compound was removed during the purification process (precipitation) and self-assembly process (dialysis against water), the concentration of Sn in the polymer micelles solution can be calculated as follows:

Table S2. The molecular mass of the material used in the synthesis polymer

material	molecular mass(g/mol)
$Sn(Oct)_2$	405.12
Sn	118.710
PEO-*b*-PCL-OH	4282.8
PEO_{43}-*b*-PCL_{20}-*b*-PTA_{20}-Br	8135.8
PEO_{43}-*b*-PCL_{20}-*b*-PTA_{30}-Br	9987.8

Maximum content (wt%) of $Sn(Oct)_2$ in PEO-*b*-PCL:

$$\frac{405.12\times 2\times 10^{-4}}{6.00+7.28}\times 100\% = 0.61\%$$

Maximum content (wt%) of Sn in PEO-*b*-PCL**:**

$$\frac{118.71}{405.12}\times 0.61\% = 0.18\%$$

Maximum content (wt%) of Sn in PEO_{43}-*b*-PCL_{20}-*b*-PTA_{20} is:

$$\frac{4282.8}{8135.8}\times 0.18\% = 0.095\%$$

Maximum content (wt%) of Sn in PEO_{43}-*b*-PCL_{20}-*b*-PTA_{30}:

$$\frac{4282.8}{9987.8}\times 0.18\% = 0.077\%$$

In PEO_{43}-*b*-PCL_{20}-*b*-PTA_{20} micelle solution:

Copolymer: 1 mM = 10^{-3} mol/L = 10^{-3} mol$\times 0.8\times 10^{4}$ g/mol/L = 8 g/L = 8 mg/mL

The concentrations of Sn in 0.28 and 0.13 mM PEO_{43}-*b*-PCL_{20}-*b*-PTA_{20} are:

0.28$\times$8 mg/mL$\times$0.095% = 2.13$\times 10^{-3}$ mg/mL = 2.13 μg/mL

0.13$\times$8 mg/mL$\times$0.095% = 9.88$\times 10^{-4}$ mg/mL= 0.988 μg/mL

In PEO_{43}-*b*-PCL_{20}-*b*-PTA_{30} micelle solution:

Copolymer: 1 mM = 10^{-3} mol/L = 10^{-3} mol$\times 10^{4}$ g/mol/L = 10 g/L= 10 mg/mL

The concentrations of Sn in 0.19 and 0.06 mM PEO_{43}-*b*-PCL_{20}-*b*-PTA_{30} are:

0.19$\times$10 mg/mL$\times$0.077% = 1.46$\times 10^{-3}$ mg/mL = 1.46 μg/mL

0.06$\times$10 mg/mL$\times$0.077% = 0.46$\times 10^{-3}$ mg/mL = 0.46 μg/mL

In reality, most of residual Sn compound should be removed during the purification process (precipitation) and self-assembly process (dialysis against water). The concentration of Sn in the polymer micelles solution should be far below 0.46 μg/mL. It is known that the antibacterial

activities of Sn compound are worse than nano silver. Even though for nano silver at a concentration of 0.46 μg/mL does not show any antibacterial activity.[2, 3]

To conclude, the antibacterial activity of polymer micelles is not from the possible residual Sn compound, but the polymer micelles themselves.

References

1. J. Z. Du and S. P. Armes, *Soft Matter*, 2010, **6**, 4851-4857.
2. W. R. Li, X. B. Xie, Q. S. Shi, S. S. Duan, Y. S. Ouyang and Y. B. Chen, *Biometals*, 2011, **24**, 135-141.
3. J. R. Morones, J. L. Elechiguerra, A. Camacho, K. Holt, J. B. Kouri, J. T. Ramirez and M. J. Yacaman, *Nanotechnology*, 2005, **16**, 2346-2353.