Development of Dual Anti-biofilm and Anti-bacterial Medical Devices

Electronic Supplementary Information

Laurence Burroughs,[a] Waheed Ashraf,[b] Sonali Singh,[c] Luisa Martinez-Pomares,[c] Roger Bayston,[b] Andrew L. Hook[a]*

[a] School of Pharmacy, University of Nottingham, Nottingham NG7 2RD, United Kingdom

[b] School of Medicine, Queen's Medical Centre, Nottingham NG7 2UH, United Kingdom

[c] School of Life Sciences, Faculty of Medicine and Health Sciences, Queen's Medical Centre, Nottingham NG7 2UH, United Kingdom

E-mail: andrew.hook@nottingham.ac.uk

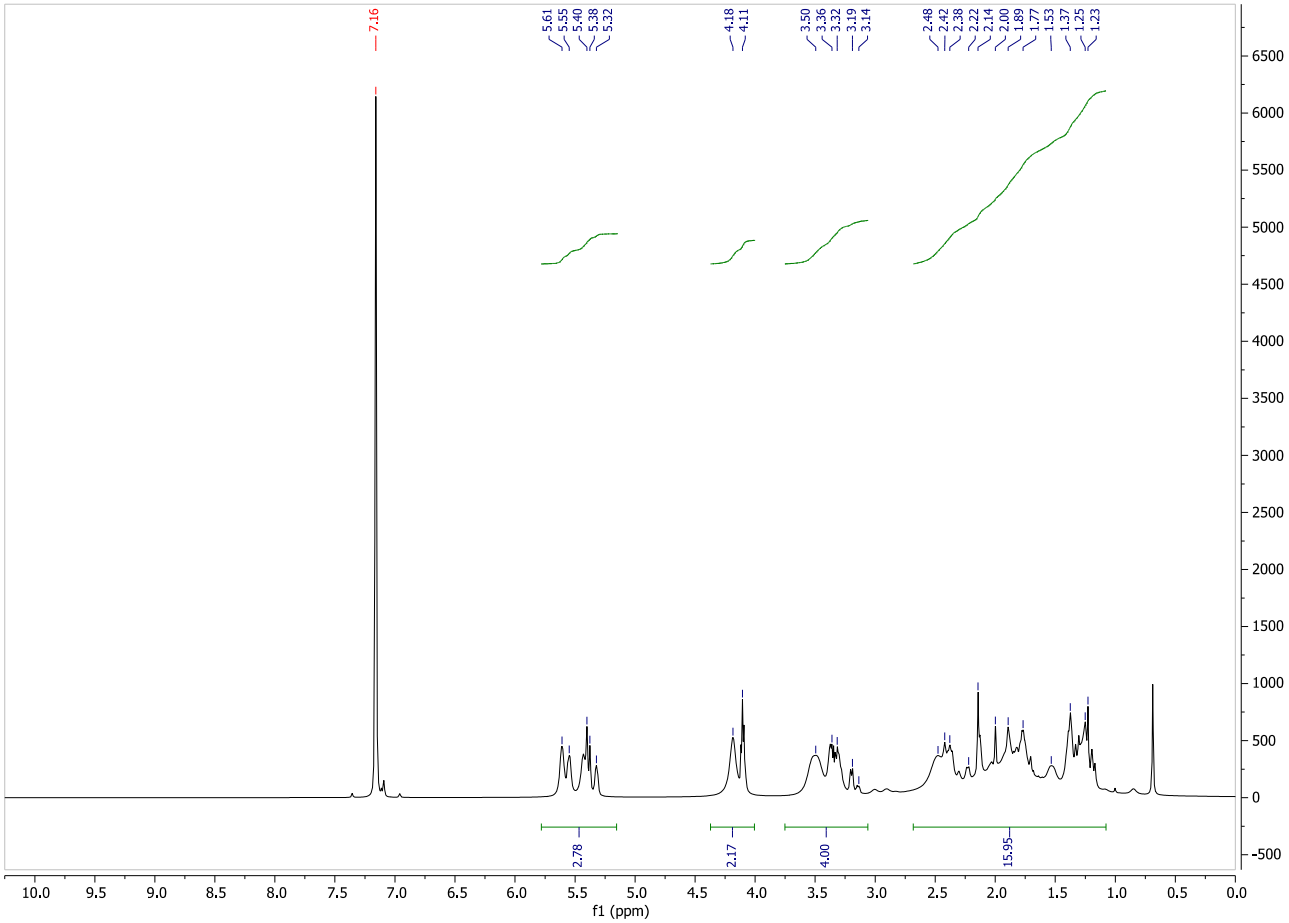


Figure SI1. NMR spectrum for p-EGDPEA.

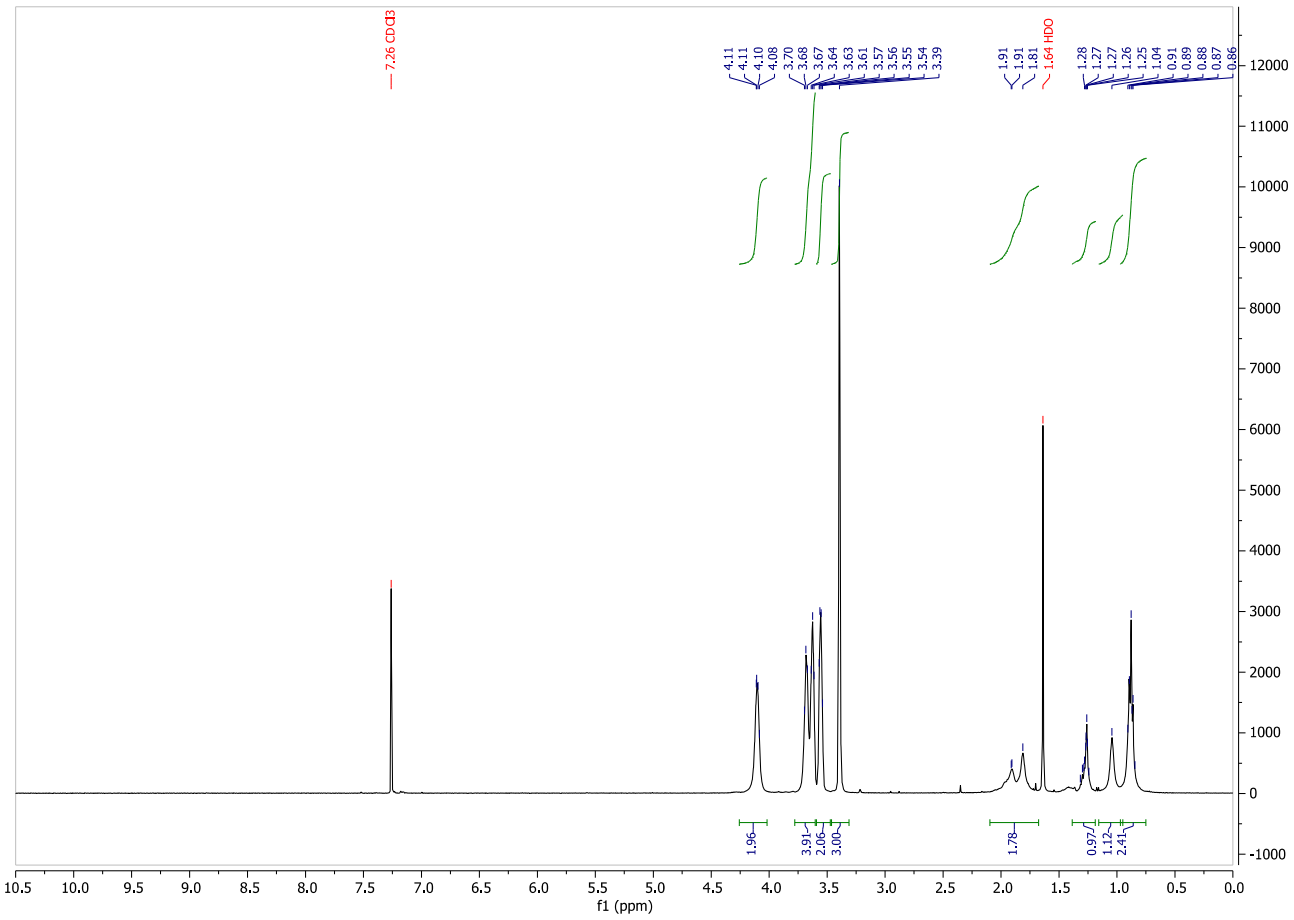


Figure SI2. NMR spectrum for p-DEGMA.

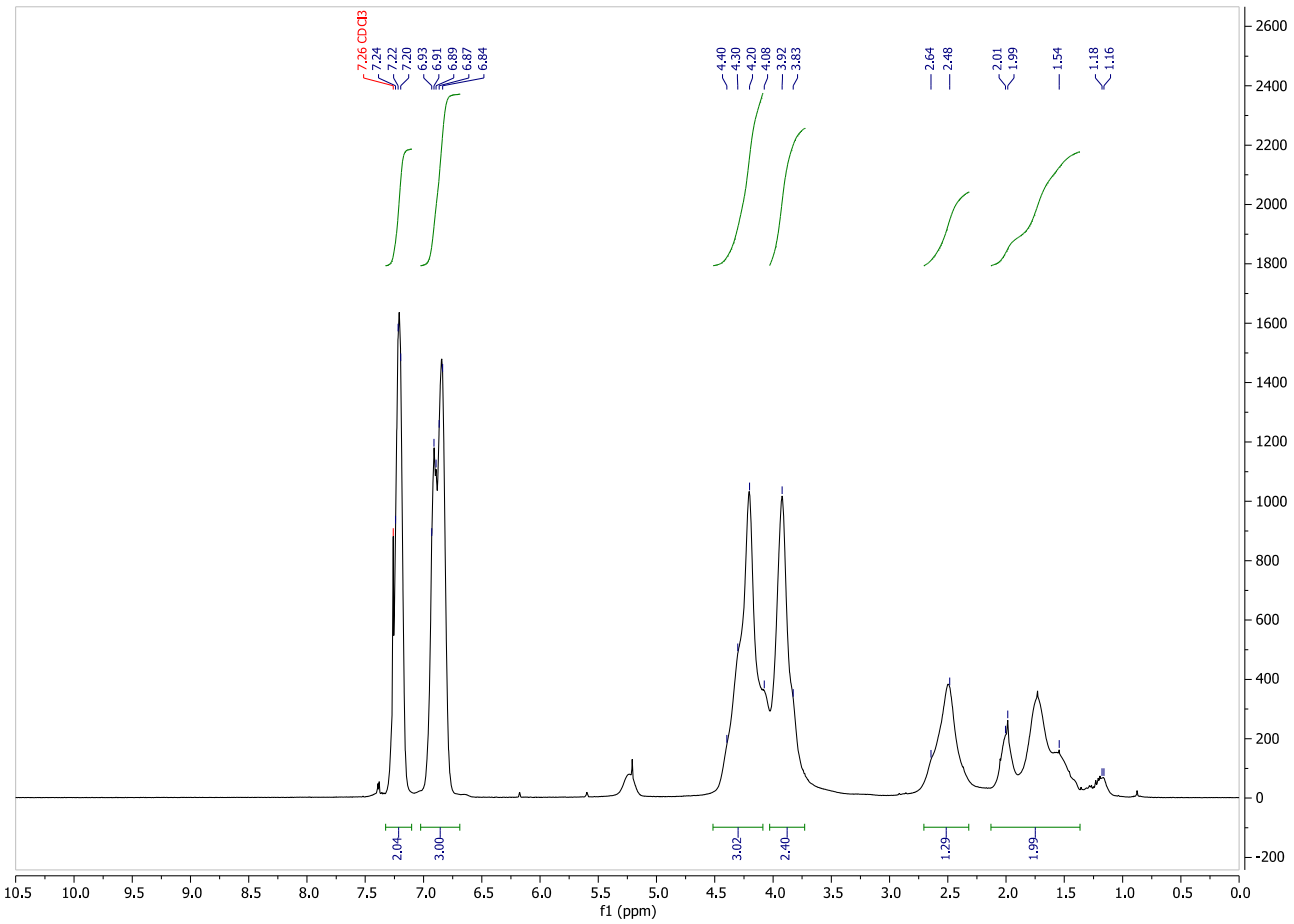


Figure SI3. NMR spectrum for p-HPhOPA.

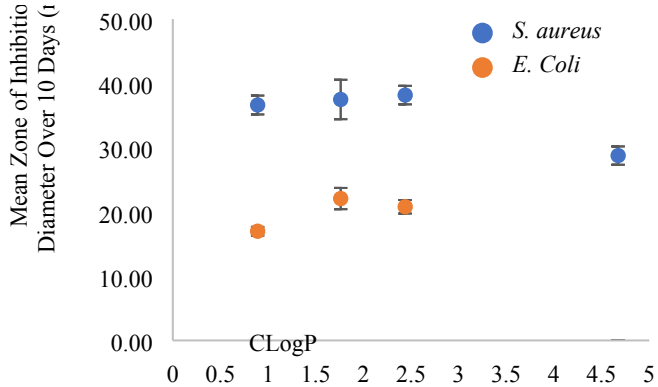


Figure SI4. Zone of inhibition measured for *S. aureus* (■) and *E. coli* (■) for various polymers with varied CLogP. Error bars equal ± one standard deviation unit, N = 3.

Optimisation of Polymer Coating

Optimisation of the p-tBCHA coating coating thickness was necessary due to the inability of this coating on an impregnated device to achieve a zone of inhibition for *E. coli.* Polymer coating was assessed in parallel as a control. Serial plate transfer tests using plates inoculated with *E. coli* were carried out on impregnated catheter sections dip-coated three times with 1 or 5 wt% polymer in toluene solutions. The zone of inhibition diameter for the two polymer solution concentrations was normalised relative to the uncoated impregnated catheter; dip-coating using the lower concentration polymer solution resulted in an increase in zone of inhibition size relative to the 5 wt% polymer solution (**Figure SI5a**). This is likely due to the low polymer concentration producing a thinner coating that only minimally inhibited antibiotic elution. The effect of polymer molecular weight on drug release was next investigated using catheter sections dip-coated with 1 wt% in toluene solutions of p-tBCHA and p-EGDPEA synthesised at various molecular weights (**Figure SI6**). The *E. coli* tK100 test was repeated using these catheter sections; both polymers showed that an optimal molecular weight of < 6.5×10^4 g mol^{-1} would permit sufficient antibiotic elution to successfully kill all attached bacteria at 72 hours (**Figure SI5b**). Tunable polymeric hydrogel systems for drug delivery have previously been reported, with polymer network and mesh size affecting drug-polymer interactions.[2] There was no difference in bacterial viability between the non-impregnated coated catheters and non-impregnated uncoated control (**Figure SI5c**), confirming that the anti-biofilm coatings were not antibacterial.

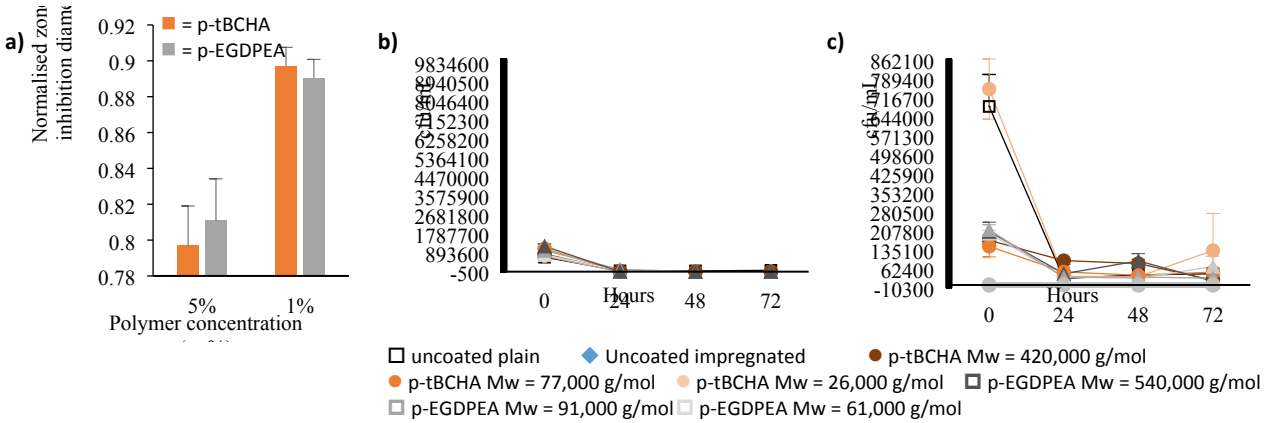
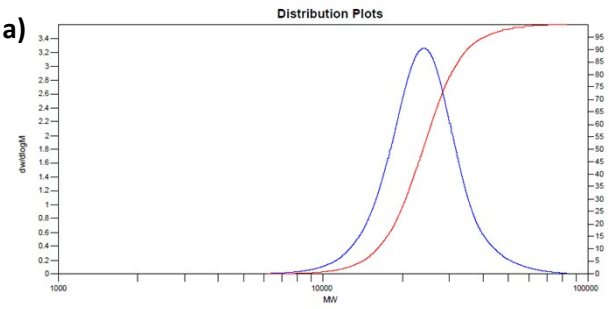
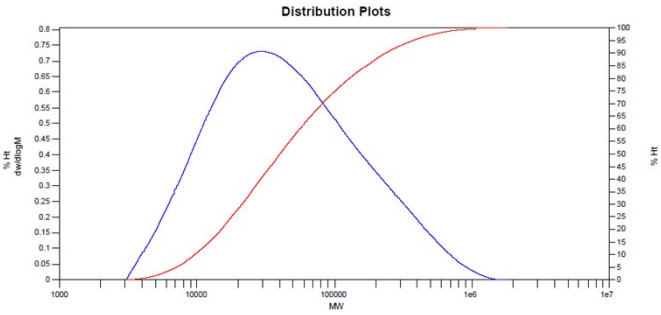


Figure SI5. Optimisation of the delivery of anti-microbials. **a)** Normalised zone of inhibition diameter (for *E. coli*) relative to uncoated impregnated catheter for varied polymer solution concentrations (wt%). Scale bars equal ± one standard deviation, n = 3. **b-c)** tK100 with **b)** impregnated and **c)** plain catheters coated with p-tBCHA or pEGDPEA at various polymer molecular weights. Scale bars equal ± one standard deviation, n = 3.

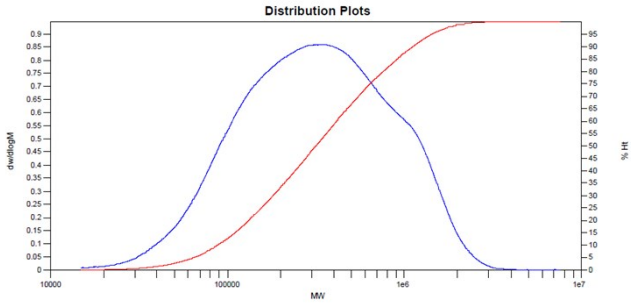
a)



Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	23535	23167	25584	28377	31799	25202	1.10433

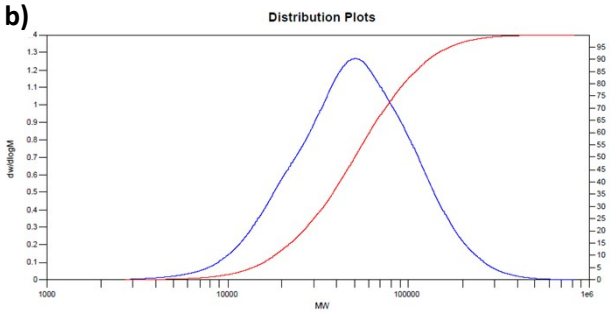


Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	37470	20364	77476	276154	529167	61737	3.80456

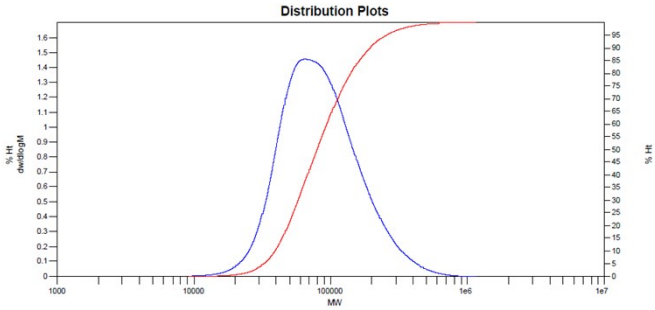


Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	417162	169130	416118	844365	1325813	366975	2.46034

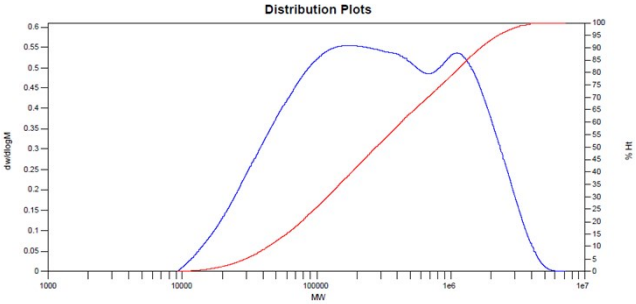
b)



Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	53183	37690	61179	97181	151030	57024	1.62322



Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	86525	64681	91441	140241	222562	86196	1.41372



Peak No	Mp	Mn	Mw	Mz	Mz+1	Mv	PD
1	1148724	100759	538254	1499357	2258428	432673	5.34199

Figure SI6. GPC traces for polymers **a)** p-tBCHA, **b)** p-EGDPEA.

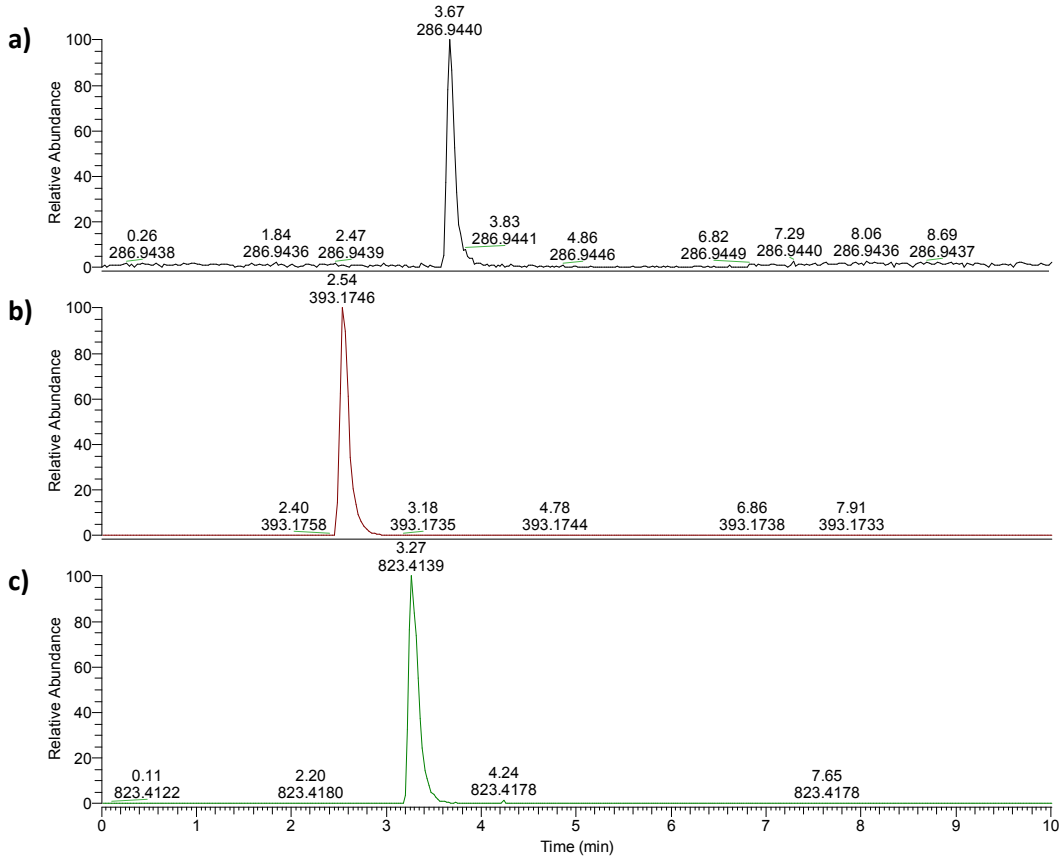


Figure SI7. LCMS spectra showing retention times (RT) and detected mass for standard solutions of **a)** triclosan RT: 3.67 min; $[M-H]^-$: $C_{12}H_6Cl_3O_2$ found 286.9440, requires 286.9433, **b)** sparfloxacin RT: 2.54 min; $[M+H]^+$: $C_{19}H_{23}F_2N_4O_3$ found 393.1746, requires 393.1738 and **c)** rifampicin RT: 3.27 min; $[M+H]^+$: $C_{43}H_{59}N_4O_{12}$ found 823.4139, requires 823.4129.

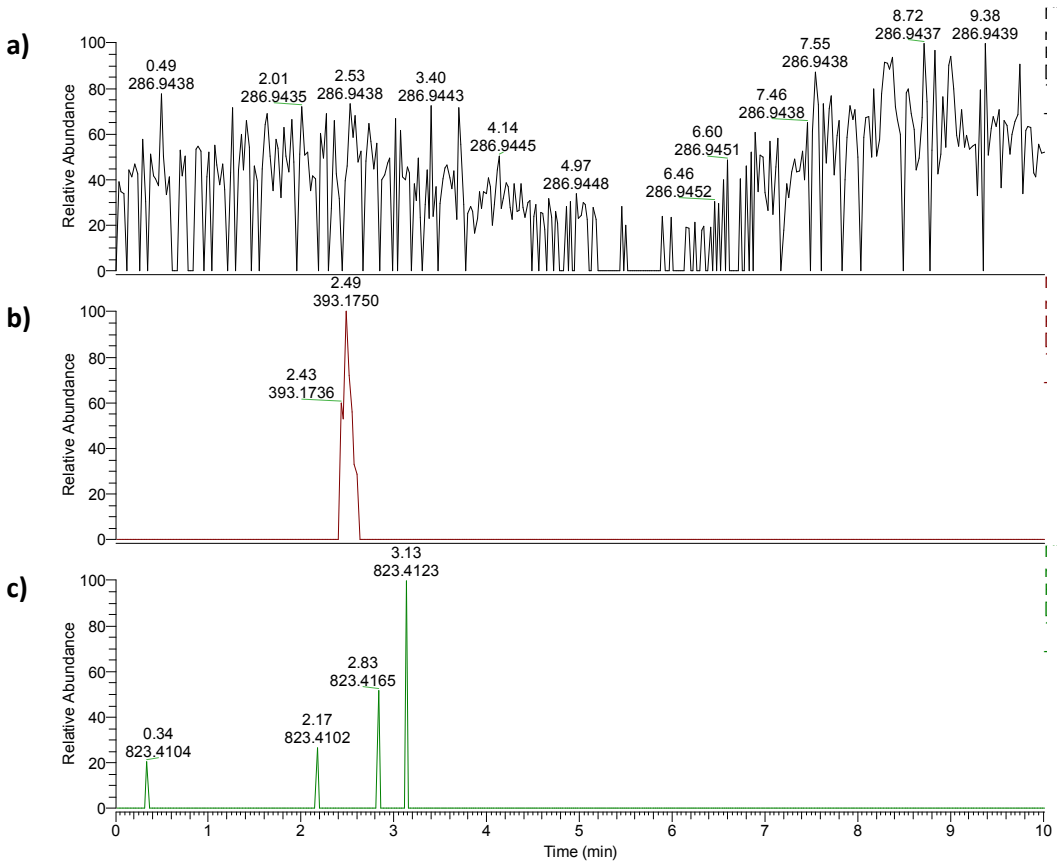


Figure SI8. LCMS spectra of the supernatant taken after incubation with a plain catheter. Ranges associated with peaks for **a)** triclosan, **b)** sparfloxacin and **c)** rifampicin assessed, as determined from standard solutions (**Figure SI7**).

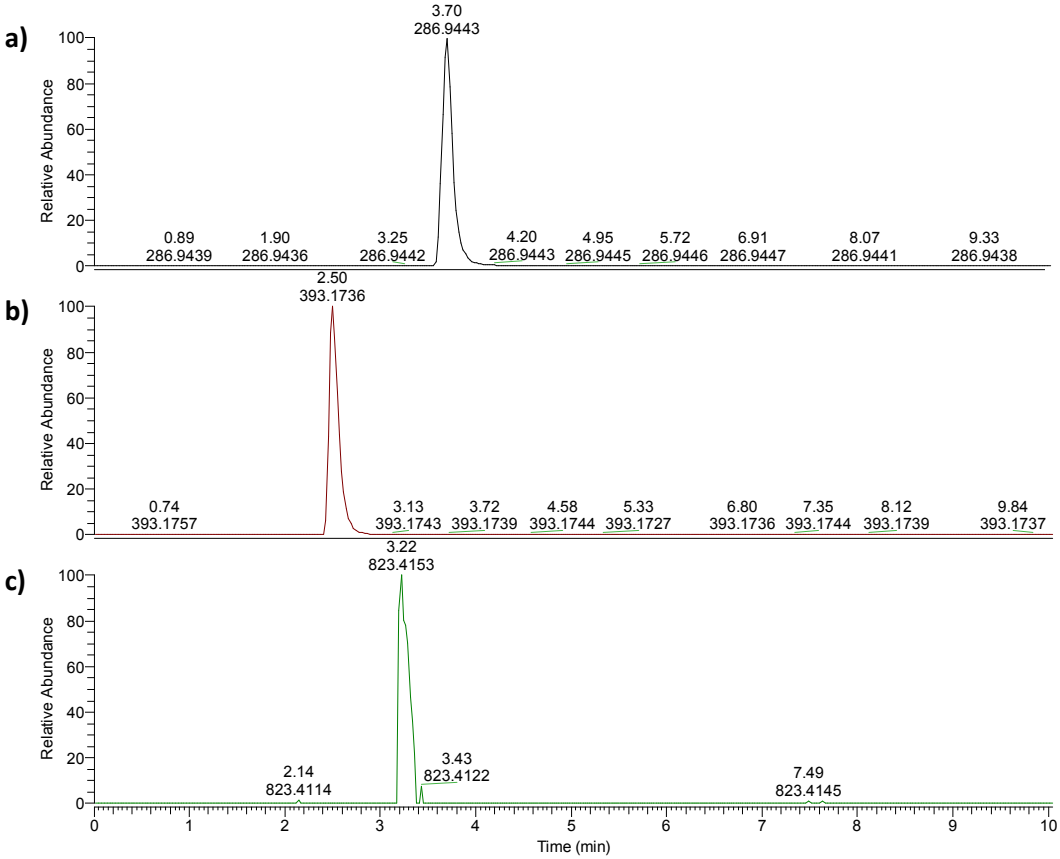


Figure SI9. LCMS spectra of the supernatant taken after incubation with an impregnated catheter coated with p-tBCHA. Ranges associated with peaks for **a)** triclosan RT: 3.70 min; $[M-H]^-$: $C_{12}H_6Cl_3O_2$ found 286.9443, requires 286.9433, **b)** sparfloxacin RT: 2.50 min; $[M+H]^+$: $C_{19}H_{23}F_2N_4O_3$ found 393.1736, requires 393.1738 and **c)** rifampicin RT: 3.22 min; $[M+H]^+$: $C_{43}H_{59}N_4O_{12}$ found 823.4153, requires 823.4129 assessed, as determined from standard solutions (**Figure SI7**).

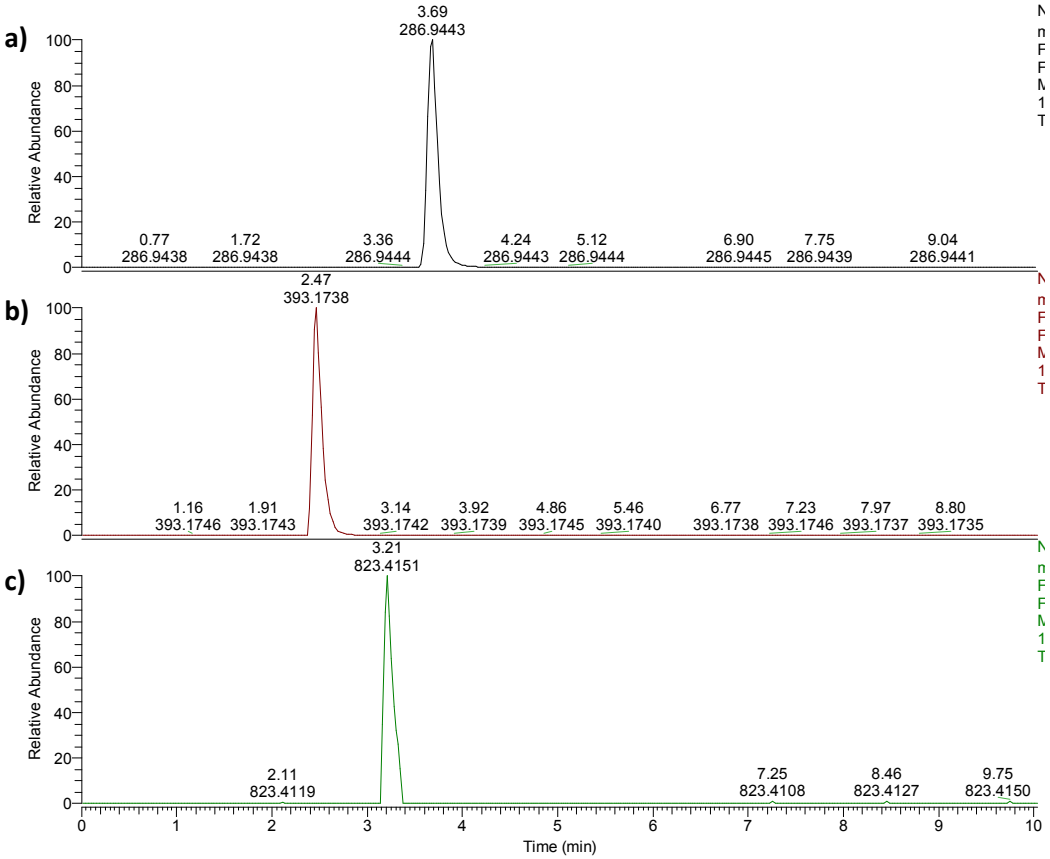


Figure SI10. LCMS spectra of the supernatant taken after incubation with an impregnated catheter coated with p-EGDPEA. Ranges associated with peaks for **a)** triclosan RT: 3.69 min; $[M-H]^-$: $C_{12}H_6Cl_3O_2$ found 286.9443, requires 286.9433, **b)** sparfloxacin RT: 2.47 min; $[M+H]^+$: $C_{19}H_{23}F_2N_4O_3$ found 393.1738, requires 393.1738 and **c)** rifampicin RT: 3.21 min; $[M+H]^+$: $C_{43}H_{59}N_4O_{12}$ found 823.4151, requires 823.4129 assessed, as determined from standard solutions (**Figure SI7**).

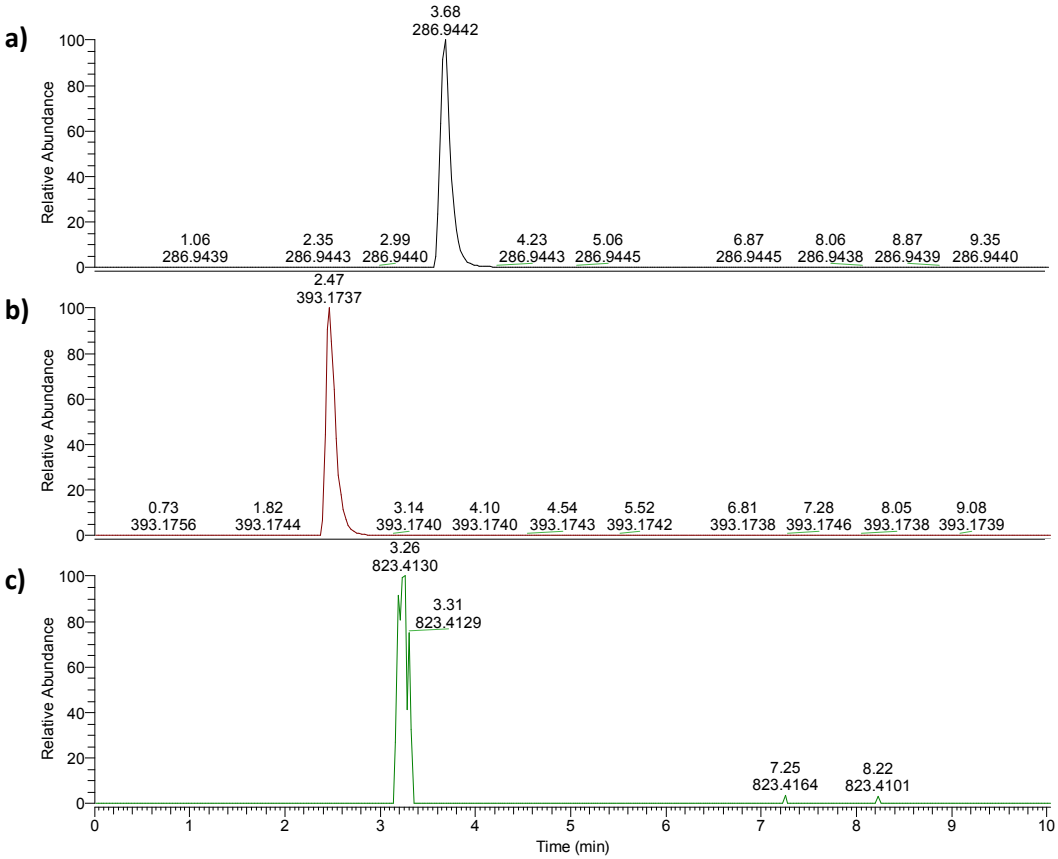


Figure SI11. LCMS spectra of the supernatant taken after incubation with an impregnated catheter coated with p-DEGMA. Ranges associated with peaks for **a)** triclosan RT: 3.68 min; $[M-H]^-$: $C_{12}H_6Cl_3O_2$ found 286.9442, requires 286.9433, **b)** sparfloxacin RT: 2.47 min; $[M+H]^+$: $C_{19}H_{23}F_2N_4O_3$ found 393.1737, requires 393.1738 and **c)** rifampicin RT: 3.26 min; $[M+H]^+$: $C_{43}H_{59}N_4O_{12}$ found 823.4130, requires 823.4129 assessed, as determined from standard solutions (**Figure SI7**).

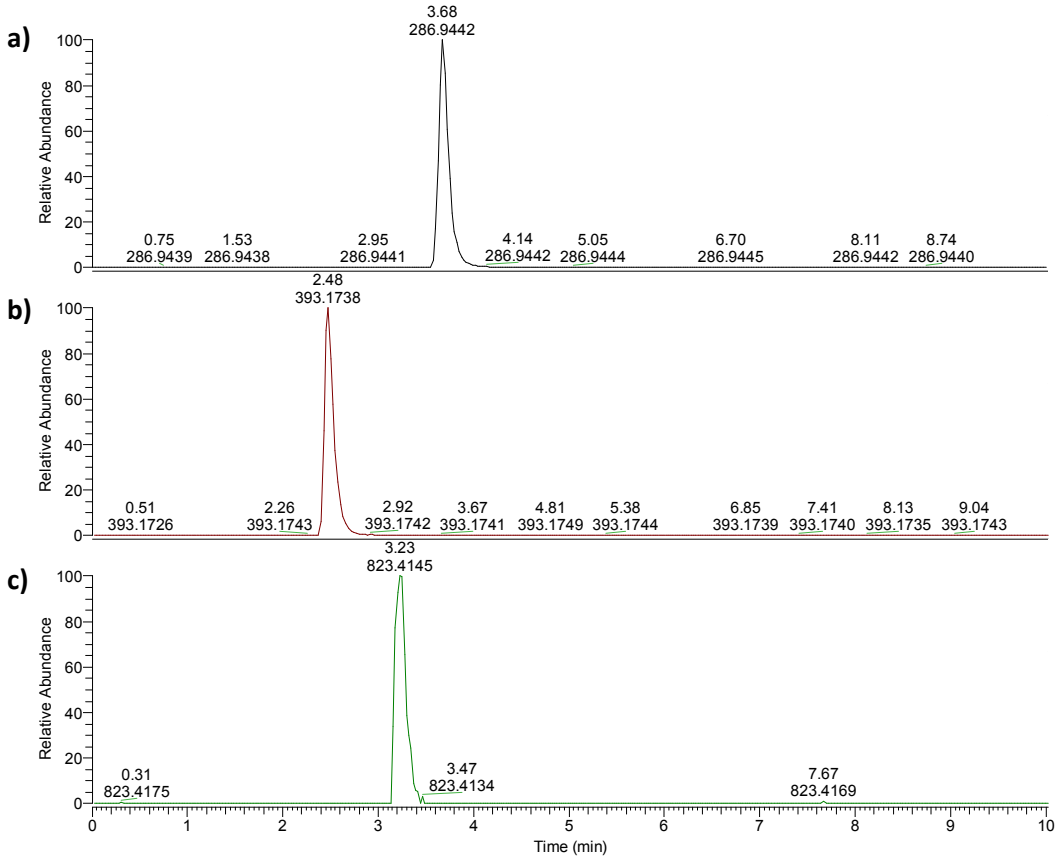


Figure SI12. LCMS spectra of the supernatant taken after incubation with an impregnated catheter coated with p-HPhOPA. Ranges associated with peaks for **a)** triclosan RT: 3.68 min; $[M-H]^-$: $C_{12}H_6Cl_3O_2$ found 286.9442, requires 286.9433, **b)** sparfloxacin RT: 2.48 min; $[M+H]^+$: $C_{19}H_{23}F_2N_4O_3$ found 393.1738, requires 393.1738 and **c)** rifampicin RT: 3.23 min; $[M+H]^+$: $C_{43}H_{59}N_4O_{12}$ found 823.4145, requires 823.4129 assessed, as determined from standard solutions (**Figure SI7**).

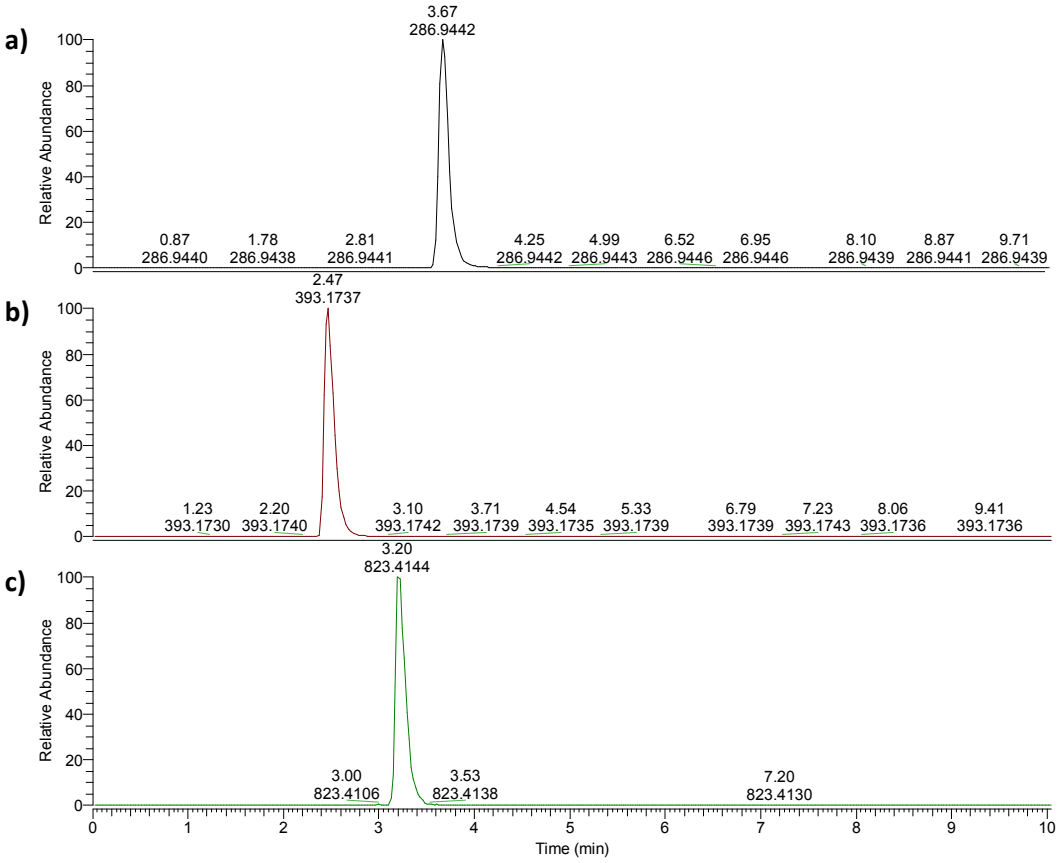


Figure SI13. LCMS spectra of the supernatant taken after incubation with an uncoated impregnated catheter. Ranges associated with peaks for **a)** triclosan RT: 3.67 min; $[M-H]^-$: $C_{12}H_6Cl_3O_2$ found 286.9442, requires 286.9433, **b)** sparfloxacin RT: 2.47 min; $[M+H]^+$: $C_{19}H_{23}F_2N_4O_3$ found 393.1737, requires 393.1738 and **c)** rifampicin RT: 3.20 min; $[M+H]^+$: $C_{43}H_{59}N_4O_{12}$ found 823.4144, requires 823.4129 assessed, as determined from standard solutions (**Figure SI7**).

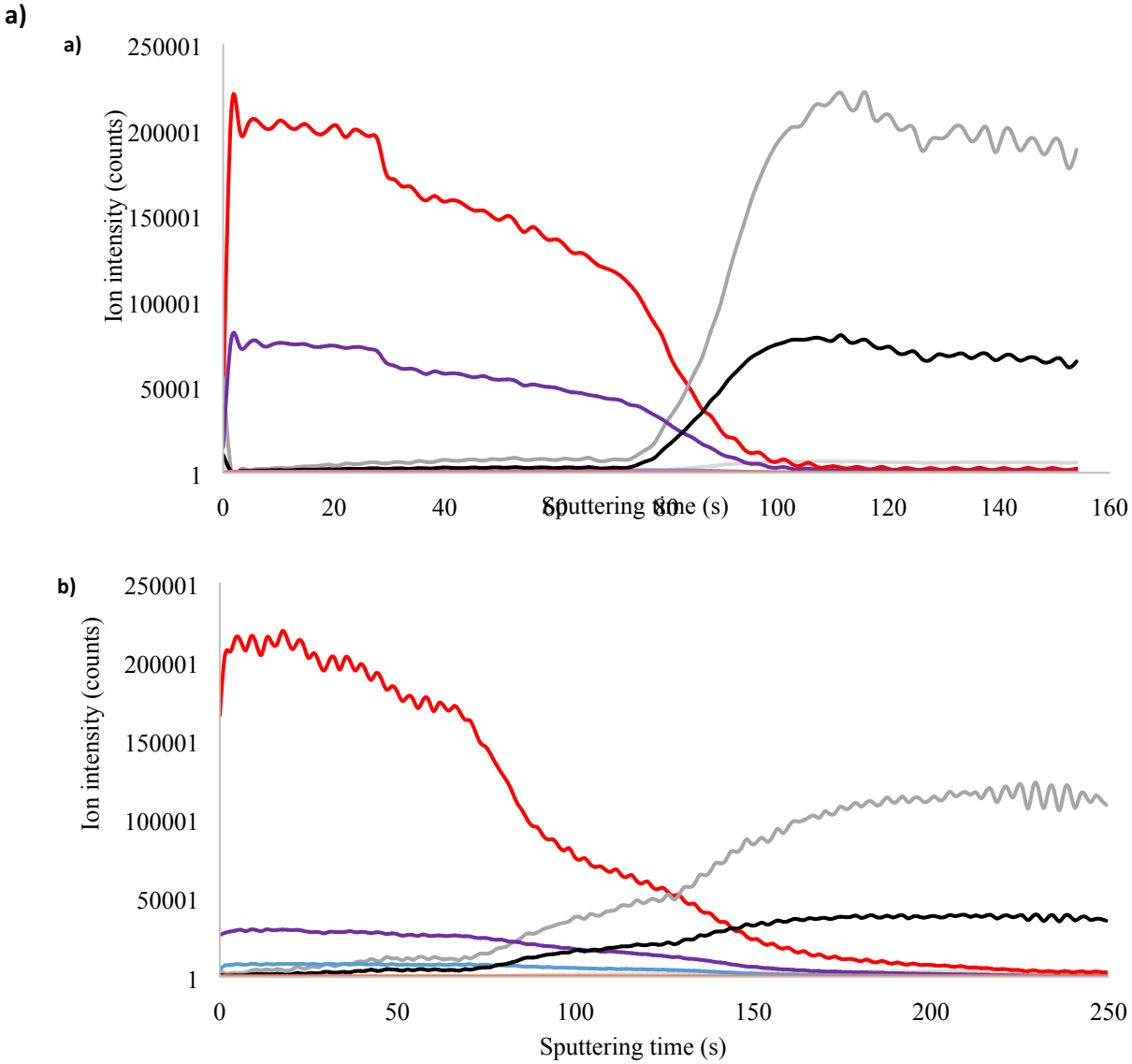


Figure SI14. Time-of-flight secondary ion mass spectrometry depth profile for **a)** p-tBCHA for ions $C_4H_9^+$ ( ), $C_6H_{11}^+$ ( ), $SiC_3H_9^+$ ( ), $Si_2C_5H_{15}O^+$ ( ), $C_5H_{11}N_2^+$ ( ), $C_{19}H_{22}N_4O_3F_2^+$ ( ), $C_{12}H_7O_2Cl_3^+$ ( ), Si^+ ( ), $C_{43}H_{58}N_4O_{12}^+$ ( ); and **b)** p-EGDPEA for ions $C_5H_7^+$ ( ), $C_7H_7^+$ ( ), $C_5H_{11}N_2^+$ ( ), $SiC_3H_9^+$ ( ), $Si_2C_5H_{15}O^+$ ( ), $C_{19}H_{22}N_4O_3F_2^+$ ( ), $C_{12}H_7O_2Cl_3^+$ ( ), Si^+ ( ), $C_{43}H_{58}N_4O_{12}^+$ ( ).

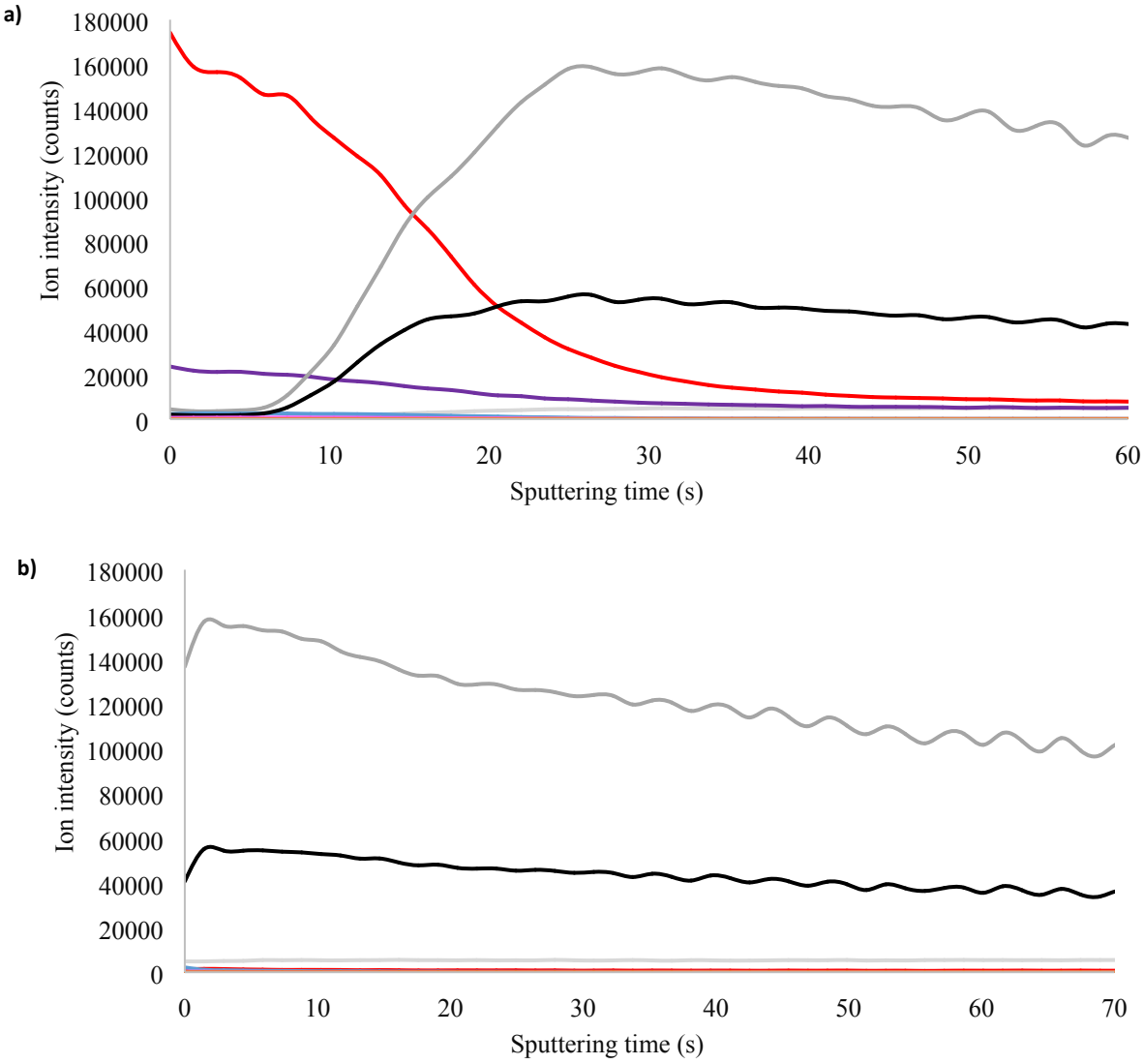


Figure SI15. Time-of-flight secondary ion mass spectrometry depth profile for **a)** p-DEGMA for ions $C_3H_7O^+$ ( ), $C_2H_5O^+$ ( ), $SiC_3H_9^+$ ( ), $C_5H_{11}N_2^+$ ( ), $Si_2C_5H_{15}O^+$ ( ), $C_{19}H_{22}N_4O_3F_2^+$ ( ), $C_{12}H_7O_2Cl_3^+$ ( ), Si^+ ( ), $C_{43}H_{58}N_4O_{12}^+$ ( ); and **b)** p-HPhOPA for ions $SiC_3H_9^+$ ( ), $Si_2C_5H_{15}O^+$ ( ), Si^+ ( ), $C_5H_{11}N_2^+$ ( ), $C_6H_5^+$ ( ), $C_3H_3O^+$ ( ), $C_{19}H_{22}N_4O_3F_2^+$ ( ), $C_{12}H_7O_2Cl_3^+$ ( ), $C_{43}H_{58}N_4O_{12}^+$ ( ).

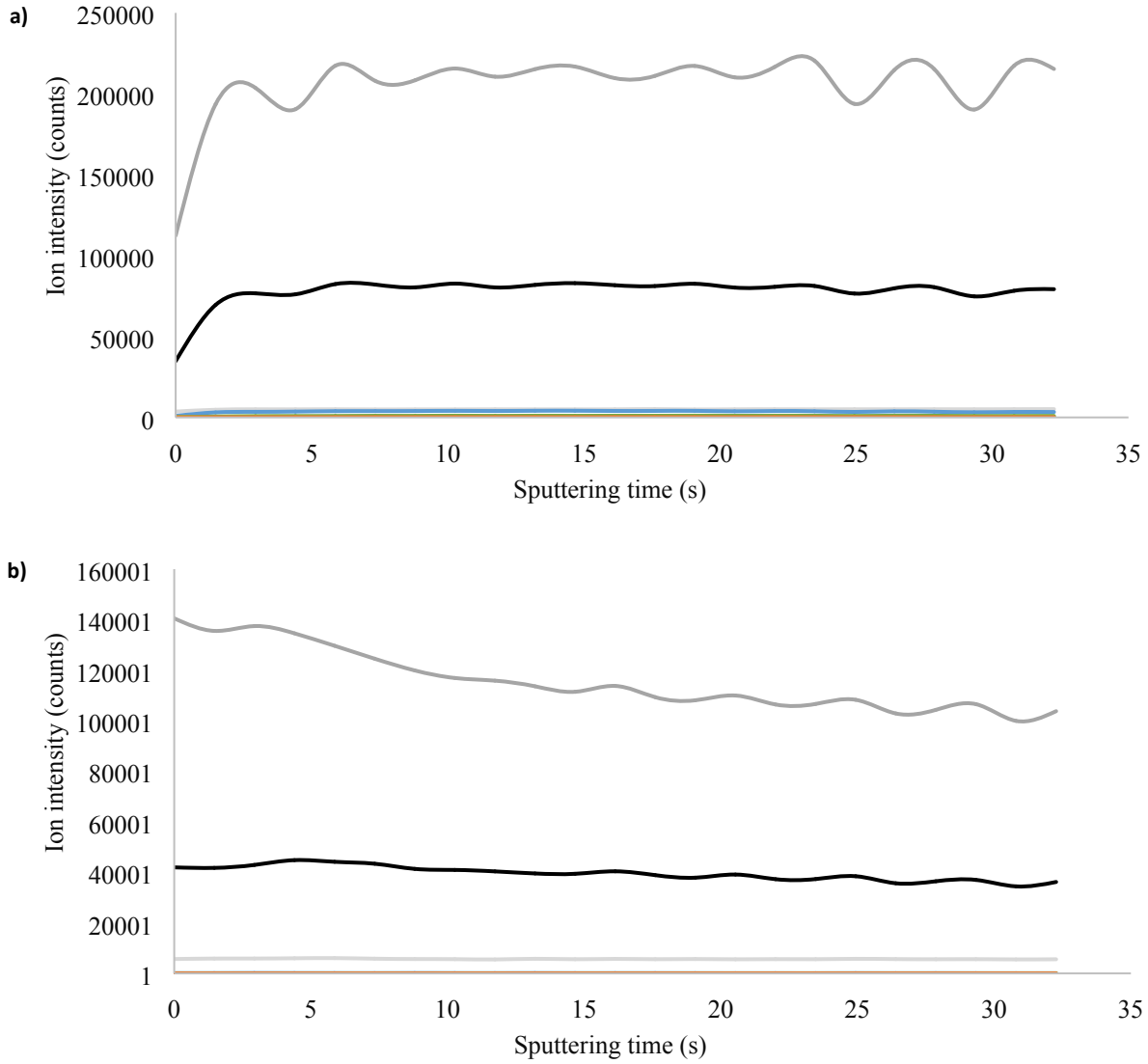


Figure SI16. Time-of-flight secondary ion mass spectrometry depth profile for **a)** an uncoated impreganated catheter and **b)** a catheter as received for ions $SiC_3H_9^+$ ( ), $Si_2C_5H_{15}O^+$ ( ), Si^+ ( ), $C_5H_{11}N_2^+$ ( ), $C_{19}H_{22}N_4O_3F_2^+$ ( ), $C_{12}H_7O_2Cl_3^+$ ( ), $C_{43}H_{58}N_4O_{12}^+$ ( ).

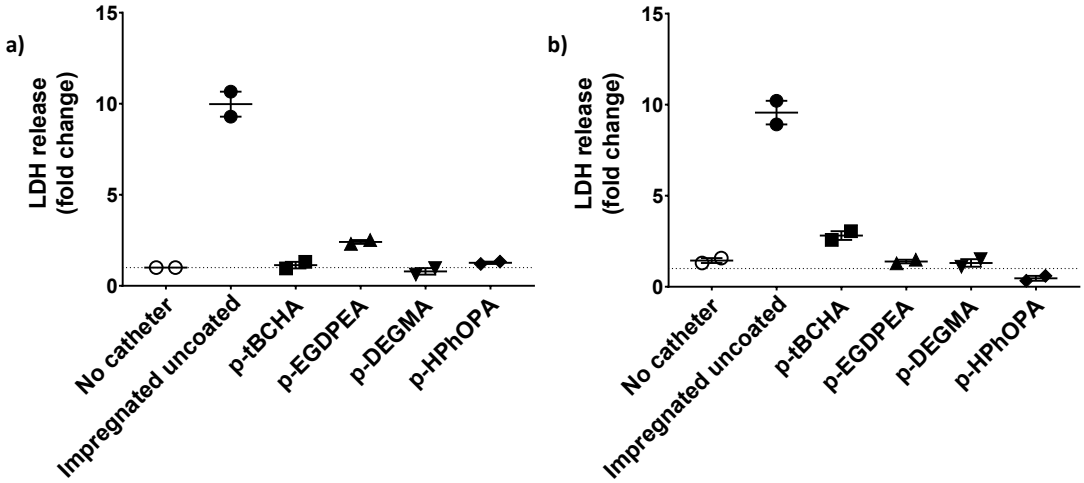


Figure SI17. Determination of macrophage viability in response to uncoated or coated antibiotic-impregnated catheters in **a)** unstmulated and **b)** LPS-stimulated conditions.[1]

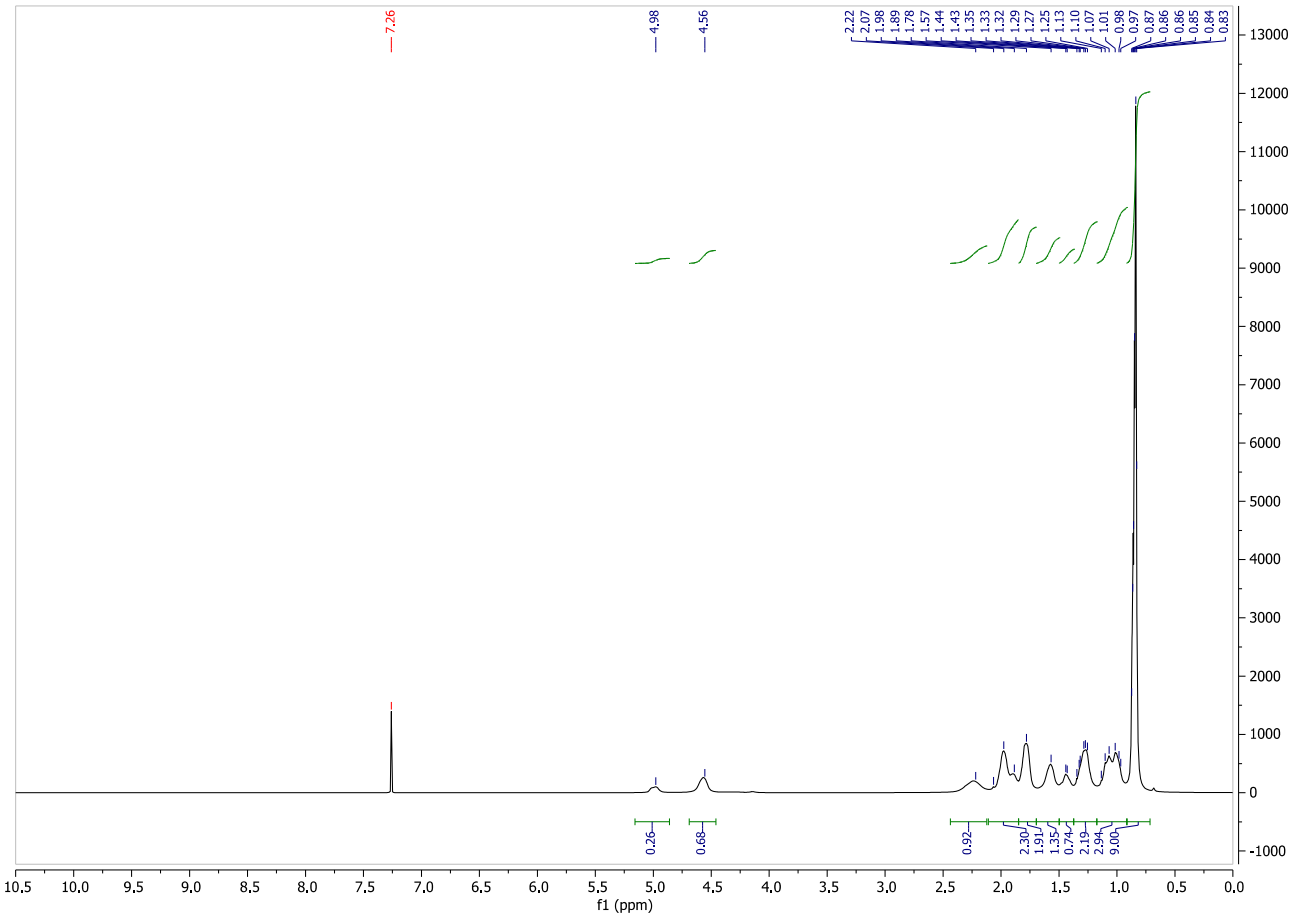


Figure SI18. NMR spectrum for p-tBCHA.

References

1 L. E. Scriven, *MRS Proceedings*, 1988, **121**, 717.

2 J. Y. Li and D. J. Mooney, *Nature Reviews Materials*, 2016, **1**.

[1] The high LDH signal observed for impreganted catheters is due, at least in part, to fluorscence associated with the antimicrobials. To gain a measure of the viability associated with the coatings, LDH measurements were undertaken on coated non-impregnated catheters.