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Non-ionic surfactants for the solubilisation of lipopeptides and enhancement of their biological activity

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Lipopeptides have been extensively explored for their antimicrobial, anticancer and hormone activity, as well as promising fully synthetic self-adjuvanted vaccine candidates. However, lipopeptides tend to violate classical medicinal chemistry filters, mainly due to their high molecular weight and LogP characteristics. Lipopeptides usually form heterogenic aggregates in the micrometre range, therefore filtration is not an appropriate method to ensure sterility of the suspension. In addition, low aqueous solubility is a challenge when performing cell-based assays and often leads to failure in drug development. Besides, the often occurring haemolytic and cytotoxic side effects of lipopeptides may also be a serious limitation for their *in vitro* and *in vivo* use.

Table 1. *Modell lipopeptides selected for this study*

Name	Composition	Molecular weight	Net charge	Biological activity
pL1	palmitoyl-LLRK	766.1	2	Short antibacterial lipopeptide
pCM15	palmitoyl-KWKLFKKIGAVLKVL	2008.6	5	Hybrid peptide with anticancer activity
pATIPC	palmitoyl-KLVANNTRLTKPK-(FFGINTIPIAC)-GGLIDIAPHQISSV	4496.8	4	Branched-chained vaccine antigen

Non-ionic surfactants: poloxamers

In this study, self-assembling triblock copolymers, namely poloxamers were investigated to control the size of aggregates and i*n vitro* properties of biologically active lipopeptides (***Table 2***). Poloxamers are non-ionic amphiphilic copolymers consisting of poly(ethylene-oxide)—poly(propylene-oxide)—poly(ethylene-oxide) blocks. Their versatility arises from their tunable properties, which are linked to the length of their hydrophilic and hydrophobic chains. They are applied in various fields, serving as drug matrices and carriers, surfactants, solubilizers, wetting agents, emulsifiers, and adsorption enhancers. Due to their biocompatibility the U.S. Food and Drug Administration (US FDA) and European Medicines Agency (EMA) have approved numerous poloxamers for human use. Moreover, polymer micelles, which are more resistant to dilution than conventional surfactants, can be produced on a large scale. Poloxamers can encapsulate molecules within the core, constructed by the hydrophobic PPO segment, while the hydrophilic PEO region acts as a protective shield. The formed micelles have small size (10-100 nm), and exhibit spherical or rod-like shapes. In this study, the below listed poloxamers were investigated and the Critical Micelle Concentration (CMC) together with the cytotoxicity effect was determined. Based on our results, three poloxamers were selected (P-104, P-123, F-127) that have relatively low CMC values and can be used in high concentrations without toxicity.

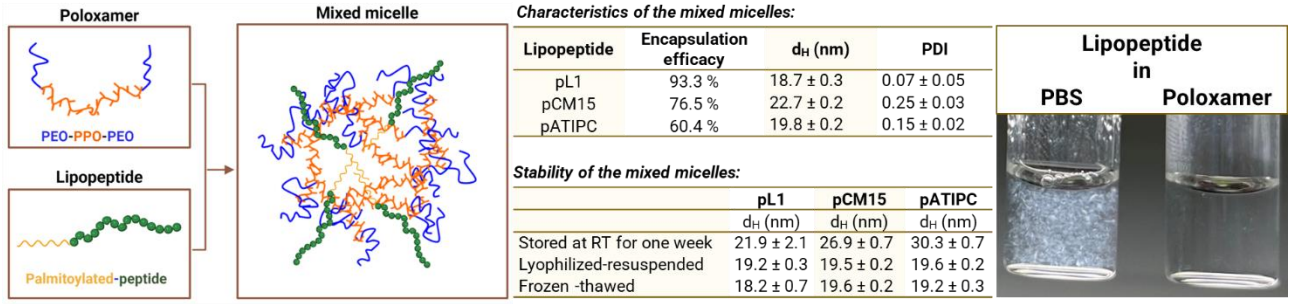
Table 2. *Characteristics of the used poloxamers*

Poloxamer name	Pluronic name	Molecular weight	EO units	PO units	HLB values	CMC literature (g/L)	CMC experimental (g/L) [1]	Cytotoxicity IC50 (g/L) [2]
334	P-104	5900	27	56	13	0.02-0.08	0.02	2.731
403	P-123	5800	20	70	8	0.01-0.02	0.02	>2.9
407	F-127	12600	97	63	22	0.03-0.2	0.08	>6.3

The state of the poloxamer is marked with liquid (L), flakes (F), and paste (P). EO: ethylene oxide; PO: propylene oxide.HLB: hydrophilic-lipophilic balance.
[1] Critical micelle concentration (CMC) values of the poloxamers at 37°C in PBS, determined by pyrene fluorescence probe method
[2] Half-maximal inhibitory concentration (IC_{50}) values of the poloxamers, measured on HepG2 human cell-line, using AlamarBlue viability assay.

Characteristics of the poloxamer – lipopeptide mixed micelles

Mixed micelles of poloxamers with model lipopeptides were prepared by a simple solvation method and through dynamic light scattering (DLS) measurements, the production of small particles (diameter of around 20 nm) and reproducible, storable systems were demonstrated. Besides, these micelles can also tolerate freeze-thaw and lyophilisation-resolution cycles.



Characteristics of the mixed micelles:

Lipopeptide	Encapsulation efficacy	d_H (nm)	PDI
pL1	93.3 %	18.7 ± 0.3	0.07 ± 0.05
pCM15	76.5 %	22.7 ± 0.2	0.25 ± 0.03
pATIPC	60.4 %	19.8 ± 0.2	0.15 ± 0.02

Stability of the mixed micelles:

	pL1	pCM15	pATIPC
	d_H (nm)	d_H (nm)	d_H (nm)
Stored at RT for one week	21.9 ± 2.1	26.9 ± 0.7	30.3 ± 0.7
Lyophilized-resuspended	19.2 ± 0.3	19.5 ± 0.2	19.6 ± 0.2
Frozen -thawed	18.2 ± 0.7	19.6 ± 0.2	19.2 ± 0.3

Improved effectivity and selectivity

During the biological assessments, the properties of lipopeptide and lipopeptide–poloxamer systems were compared. The results showed that selectivity of pL1 lipopeptide was significantly improved by the poloxamers, increasing its antibacterial activity while reducing its haemolytic activity. The cytostatic effect of pCM15 colorectal tumor cells in the presence of poloxamers was approximately twice as high as that of the unformulated lipopeptide. For the self-adjuvanted vaccine antigen pATIPC, the rate of cellular uptake by antigen presenting model cells increased several-fold in the presence of poloxamers. Among the poloxamers, the highest biological activity was observed when Plur123 was used.

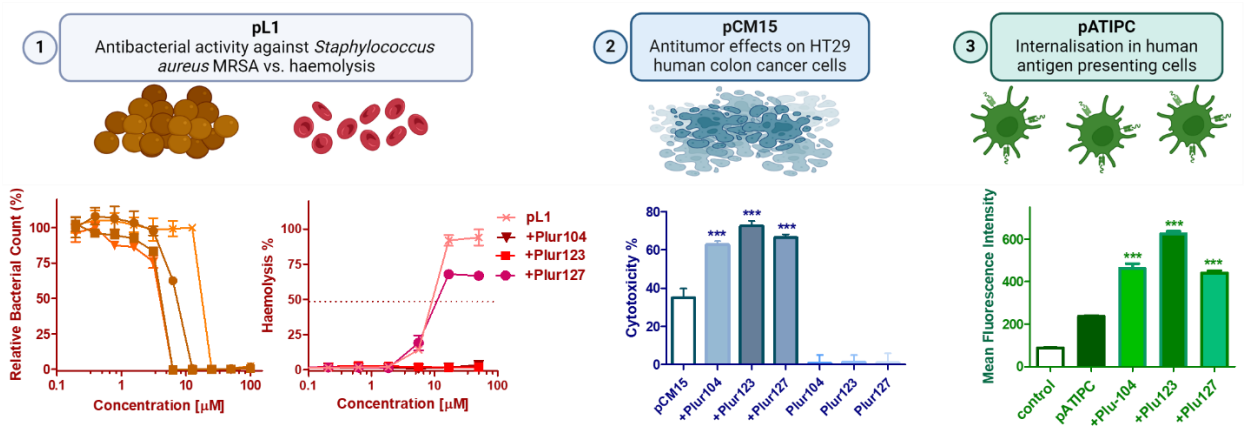


Figure 1. *Biological effect of the unformulated lipopeptides ans their mixed micelles with poloxamers*

Overall, it is concluded that poloxamer formulation is effective in optimizing the effect of lipopeptides, but the final properties of the mixed micelles are highly dependent on the length and structure of the peptide chain. It is believed that the optimal peptide chain length that can still be hidden in the PEO chains of Plur123 is around 10–12 amino acids.

Acknowledgements
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Reference
Ábrahám, Á. et al. Optimizing lipopeptide bioactivity: The impact of non-ionic surfactant dressing. *J Pharm Analysis*, 2024, 101020.