COMMUNICATION

Using Pyrene to Probe the Effects of Poloxamer Stabilisers on Internal Lipid Microenvironments in Solid Lipid Nanoparticles

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Solid lipid nanoparticles (SLNs) have proved to be effective nanocarriers with many advantages over other non-lipid-based systems. The development of new SLN formulations is often hindered through poor drug loading capacity and time-consuming optimisation of lipid/stabiliser combinations. Herein, we report the use of pyrene to probe the internal lipid microenvironment inside SLNs. We investigate the effect of using different poloxamer stabilisers on the internal polarity of SLNs formied using the common solid lipid, Compritol 888 ATO. We show that the internal lipid environment is controlled by the length of the poly(propylene oxide block) of poloxamer stabiliser, with longer blocks producing SLNs with less polar lipid cores. Blending of stabilisers could also be used to tune the polarity of the core lipid environment, which may allow for adjusting the polarity of the lipid to assist the loading of different therapeutics.

Nanomedicine has offered the potential to improve the pharmacological profiles of poorly performing or poorly soluble medicines.[1] Solid lipid nanoparticles (SLNs) are a type of nanomedicine that is composed of a lipid core that remains solid at body temperature and an active pharmaceutical ingredient (API).[2–4] The hydrophobic lipid/API cores are stabilised by amphiphilic stabilisers such as poloxamers (often known by the brand names Pluronics® or Synperonic®), which are triblock copolymers of polyethylene oxide-polypropylene oxide-polyethylene oxide blocks (PEO-PPO-PEO) (Figure 1).[5,6] They have shown a high degree of versatility as stabilisers due to their low biological toxicity and ability to enhance the solubilisation of lipophilic compounds.[7] They have improved pharmacological profiles of several poorly performing small molecule drugs and are the most common stabilisers used in SLN formulations.[5,6] Compritol 888 ATO is one of the most commonly lipids used within SLNs.

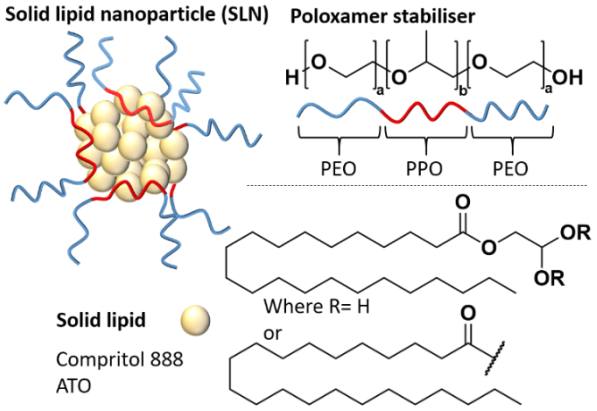

Figure 1: Solid lipid nanoparticles (SLNs) composed on an amphiphilic stabiliser (in this case poloxamers) and a solid lipid core. Poloxamers are triblock copolymers of polyethylene oxide-polypropylene oxide-polyethylene oxide blocks (PEO-PPO-PEO). The more hydrophobic PPO block will preferentially interact with the lipid core while the PEO blocks extend into the continuous phase and provide steric stabilisation. Compritol 888 ATO is a common solid lipid and is made of a mixture of mono, di and triglycerides of behenic acid.

This solid lipid is a mixture of mono, di and triglycerides of behenic acid (Figure 1), has been well documented showing high encapsulation efficiencies of still several hydrophobic entities including triamcinolone acetonide (99%), acyclovir (56-81%), natamycin (85%) and indomethacin (72%).[8–12] Interestingly, all of these formulations also share a mutual secondary excipient, a poloxamer, Pluronic® F68 as an amphiphilic stabiliser. It is known that the incorporation of APIs within the core of SLNs disrupts the natural crystallinity of the lipid, potentially facilitating a more controlled, site specific release of the API.[13–16] SLNs can be produced by a number of methods including hot, cold or high speed homogenisation, ultrasonication, spray drying and nanoprecipitation.[17] Of these methods, nanoprecipitation is a particularly attractive method due to practical simplicity and scalability, as well as time and cost effectiveness.[18–22] Nevertheless, there are still some challenges to address in the development of SLNs. A common drawback is as a result of low drug loading capacity compared to some other nanocarrier systems.[13,23–25] This limitation is typically associated with poor compatibility between the crystalline lipid and the API mixture resulting in phase separation in the core.[13,15,26] To address these drawbacks,

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considerable optimisation of the SLN formulation is typically required involving the screening different compositions and stabilisers, which typically involve time-consuming trial and error methods. It is known that the stabilisers can influence the internal polarity of the environment inside nanoparticles, which might influence the drug loading behaviour.[27] Thus, there is a need obtain an understanding how the selection of stabilisers influences the polarity of the lipid core microenvironment. This understanding may be used to enhance the lipid/API affinity and reduce API partitioning. For example, if the relevant API is highly hydrophobic, the lipid environment should be hydrophobic also; however, if the API contains some polar character, then the lipid environment might be tuned to accommodate a more polarity in the lipid core microenvironment. Changes in polarity of the core environment can be observed through implementing pyrene as a fluorescent probe. Pyrene is a small molecule that has a unique, polarity dependent emission spectra, that consists of five defined vibrational emission bands (I_1-I_5). The first polarity dependent band (I_1) and the third polarity independent band (I_3), gives a ratio (I_1/I_3), which fluctuates to a lower value with an increase of hydrophobicity.[5,28–32] As pyrene is highly hydrophobic, it partitions into the lipid phase in SLN systems and therefore provides relevant information on the polarity of the lipid core and the effect that different stabilisers have on this environment.

Once an understanding is developed of how stabiliser properties influence the polarity of the SLN cores, this knowledge can then be translated to maximise drug loading and chose stabilisers suited to the lipid/API combination.

In this study, we have chosen to investigate the influence of poloxamer stabilisers and their effect on the polarity of the core made of the common solid lipid, Compritol 888 ATO (Figure 1). Within the family of poloxamer stabilisers (>50 polymers) there is huge variability in the hydrophilic-lipophilic balance (HLB) values, molecular weight and PPO/PEO ratios. We have therefore selected four poloxamers, commonly used in SLN formulations, (Pluronic® P105, F127, L64 and F68) to compare the influence of their HLB, molecular weight and PPO/PEO ratio (Table 1) on their ability to produce stable SLNs and their influence on the polarity of the SLN core microenvironment.

Firstly, blank SLNs were prepared in triplicate to show reproducibility and allow comparison of all physical properties of the solid lipid nanoparticles with and without pyrene. The following nanoprecipitation method was used; Compritol 888 ATO (18 mg, 4.5 w/v%) was heated to 82°C in 1-propanol (4 mL) for 5 minutes. The aqueous phase (20 mL) containing the poloxamer stabiliser (16 mg, 0.8 w/v %) was agitated using mechanical stirring at 350 rpm and warmed to 26°C. The heated hydrophobic phase was then rapidly injected into the aqueous phase and stirred for a further 5 minutes. To obtain pyrene loaded particles, the method was repeated where pyrene (0.34 mg) was incorporated into the hydrophobic lipid phase in 1-propanol prior to injection. These methods resulted in either blank SLNs with a mass composition of Compritol 888 ATO (53 wt%) and poloxamers stabilisers (47 wt%), or pyrene-SLNs containing an additional 0.34 mg of pyrene at 0.1 wt% of the total solid mass. All nanoprecipitations resulted in a dispersion in a water and 1-propanol mixed solvent system (5:1). The particles were analysed by dynamic light scattering (DLS) in order to determine the size distribution and mean diameter. Fluorescence measurements were undertaken to assess the polarity of the pyrene probe. For full experimental details see the electronic supplementary information (ESI), Section E1.

Each poloxamer was investigated for its effect on mean particle diameter (D_z), polydispersity index (PdI) and changes in internal core polarity. Particle size analysis showed monomodal particle size distributions for both the blank and pyrene-loaded SLNs. All SLNs had very similar mean diameters, 230 ± 27 nm for unloaded SLNs**,** and 242 ± 48 nm pyrene-loaded SLNs regardless of the poloxamer used as the stabiliser (Figure 2 and ESI, Figure S1). This therefore revealed that the different poloxamer stabilisers had little influence on formation of the SLNs and that there was no significant difference in particle size upon the incorporation of pyrene. This meant that any differences in the polarity of the SLNs prepared with the different stabilisers would be due to the environment inside the lipid core rather than differences in the size of the particles.

Table 1: The poloxamers stabilisers chosen to investigate their effect on lipid core polarity. This table contains cited HLB and CMC data from Figueiras et al. [33] Poloxamers are ordered in increasing I_1/I_3 values with respect to SLN dispersions.

	Poloxamer (Pluronic®)	Formula	Average Mw (g/mol)	CMC at 25°C (M)	HLB value	PPO/PEO ratio	I_1/I_3 Pluronic®-micellar solutions	I_1/I_3 SLN dispersions	*e/m* ratio of SLN dispersions
1	P105	PEO_{37}-PPO_{56}-PEO_{37}	6500	6.2×10^{-6}	15	0.76	1.63 ± 0.07	1.30 ± 0.02	0.18
2	F127	PEO_{100}-PPO_{65}-PEO_{100}	12600	2.8×10^{-6}	22	0.33	1.66 ± 0.01	1.32 ± 0.03	0.21
3	F68	PEO_{76}-PPO_{29}-PEO_{76}	8400	4.8×10^{-4}	29	0.20	1.72 ± 0.02	1.39 ± 0.005	0.17
4	L64	PEO_{13}-PPO_{30}-PEO_{13}	2900	4.8×10^{-4}	15	1.2	1.70 ± 0.001	1.40 ± 0.01	0.15

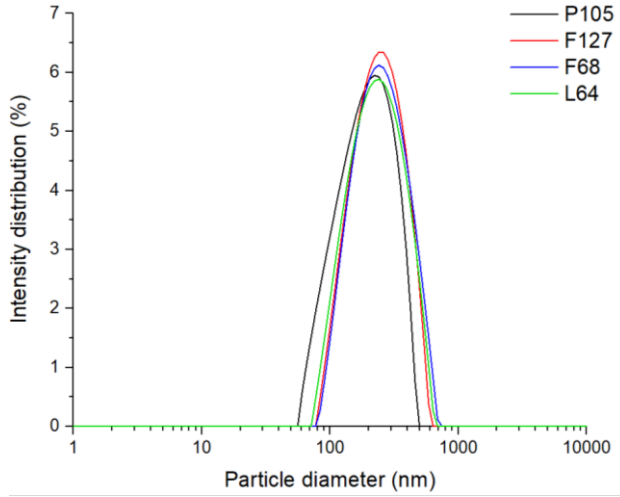

Figure 2: The monomodal size intensity distributions of pyrene loaded solid lipid nanoparticles.

The fluorescence emission behaviour of pyrene and its link to polarity was then investigated with the comparison of three different solvent environments: pure water, water and 1-propanol mixed (5:1) (the environment formed after nanoprecipitation) and pure 1-propanol. (ESI, Figure S2). The resulting I_1/I_3 values showed the differences in the polarities provided by the different solvents with values of 2.18 ± 0.09, 1.78 ± 0.003 and 1.21 ± 0.002 for pure water, water and 1-propanol mixed (5:1) and pure propanol respectively, showing that increased water content would increase the polarity around the pyrene molecules. All subsequent samples were measured with a mixed solvent continuous phase of water and 1-propanol mixed (5:1). The effect of the different poloxamers on polarity environment was then assessed. Firstly, nanoprecipitations of pyrene were carried out into the poloxamer solutions without lipid present to give forming micellar solutions. All the poloxamers were used at the same concentration (0.8 mg/ml) as used for SLN preparation. The resulting micellar solutions were significantly less turbid than SLN dispersions (ESI, Figure S3). Comparison of the I_1/I_3 values for pyrene showed that all micelles of the different poloxamers had values ranging from 1.63-1.72 (ESI, Figure S4), slightly less polar than the mixed solvent continuous phase itself. This result indicated that some of the pyrene had partitioned into the hydrophobic PPO cores of the micelles. Out of the four different poloxamers, F68 and L64 the two stabilisers with the shortest PPO blocks (Figure 3A) showed the most polar internal environments (Figure 3B). Pyrene-loaded SLNs were then prepared using the four different poloxamer stabilisers and their I_1/I_3 values for pyrene-loaded SLNs prepared were recorded. The incorporation of the lipid resulted in a considerable reduction in the I_1/I_3 values to 1.30-1.40 for all poloxamer stabilisers compared to the micellar solutions (Figure 3B).

The significant difference in the pyrene fluorescence between aqueous micellar solutions and SLNs can be attributed to the hydrophobic nature of Compritol 888 ATO and its ability to entrap hydrophobic molecules, in between the C_{22} alkyl chains.[34–36] As with the I_1/I_3 data for the micelles, it was clear that there were noticeable similarities in the I_1/I_3 values for F68 and L64 compared to F127 and P105 both in the micellar solutions and in SLN dispersions. The PPO blocks of the poloxamers will adsorb and interact with the lipid core, while the PEO chains will extend into the continuous phase and provide steric stabilisation. It was apparent that the molecular weight of the hydrophobic PPO units is a dominant factor for controlling the polarity inside the lipid cores of the SLNs. The poloxamers with longer hydrophobic blocks, F127 (PPO= 65) and P105 (PPO= 56) showed significantly lower I_1/I_3 ratios in comparison to poloxamers with shorter PPO blocks, L64 (PPO= 30) and F68 (PPO= 29). This change in polarity can be attributed PPO chains partitioning into the lipid core and reducing the internal polarity. While F127 possess a longer PPO block compared to P105, it did not have the lowest average I_1/I_3 value at 1.32 ± 0.03 *vs* P105 at 1.30 ± 0.02. This can potentially be attributed to the much larger PEO block of F127 (PEO=200) compared to P105 (PEO=74) causing increased steric hindrance between the F127 units which might potentially limit the extent to which PPO block of F127 can associate with the lipid core. This result showed that the polarity inside the lipid cores of the SLNs was being controlled by the length of the PPO block of the poloxamer.

The hydrophilic-lipophilic balance (HLB) is used often used to predict the properties and potential uses of stabilisers.[37] As shown in Table 1, Pluronic® P105 and L64 have the same cited HLB value of 15.[33] However, P105 and L64 stabilised SLNs had significantly different I_1/I_3 values of 1.30 ± 0.02 and 1.40 ± 0.01 and therefore it can be concluded the HLB is not a viable parameter to predict microenvironment polarity. This suggests that there was very limited incorporation of PEO component of the stabilisers into the lipid core; if there was greater incorporation of the PEO component of the poloxamer then the I1/I3 value should have been highest for the stabilisers with the highest HLB value. The difference in the I_1/I_3 values could potentially be a result of the average fluorescence signal from the pyrene encapsulated in the lipid cores of the SLNs and any pyrene encapsulated in the micelles. In order to investigate this the samples were centrifuged and the supernatant was analysed in the absence of the SLNs (ESI, Figure S5). Any fluorescence from the supernatant would represent the pyrene contained in the micelles. However, the supernatant was found to display very limited fluorescence with ill-defined emission spectra. This finding revealed that the pyrene was predominantly encapsulated within the lipid cores of the SLNs independent of the stabiliser used.

The difference in lipid/stabiliser behaviour of these SLN samples was also apparent from the excimer emission which was observed at 440-500 nm in the pyrene emission spectra (Figure 3C). This is a fundamental feature in pyrene fluorescence with reported intermolecular spatial proximity within ≤3.5 Å.[38] The poloxamers F127 and P105 (those with the longest PPO blocks) exhibited more intense excimer emissions signifying a stronger spatial proximity to other pyrene monomers within the lipid core, coupled with a higher excimer/monomer (*e/m*) ratio (Table 1).[15]

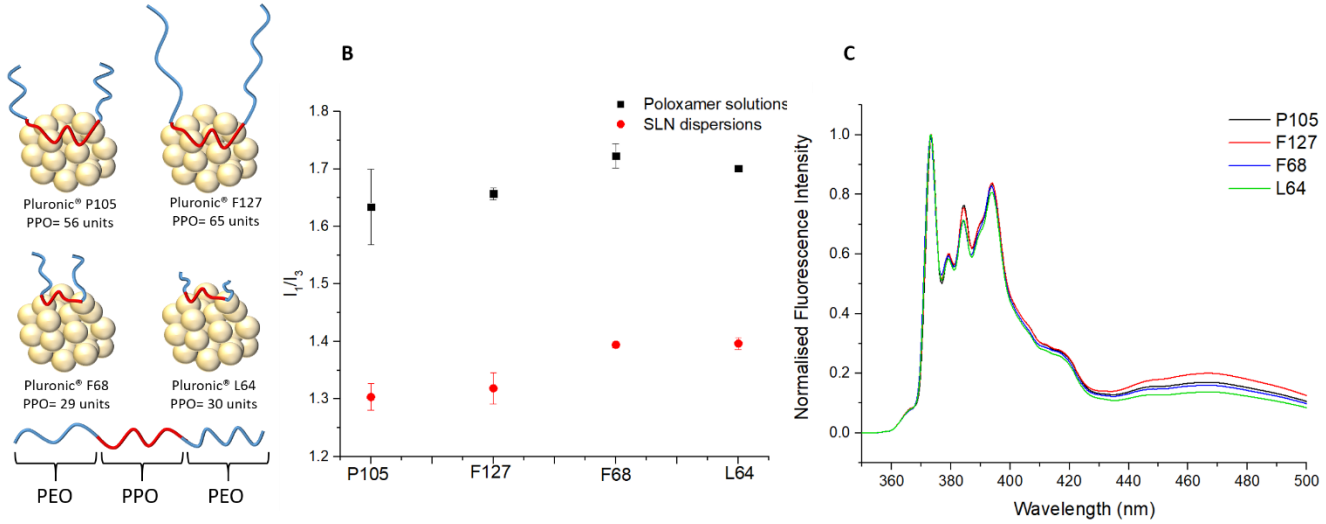

Figure 3: The fluorescence emission behaviour of SLNs with different poloxamer stabilisers. (A) Schematic representation on the different poloxamers and their interaction with a lipid core. (B) Comparison of I1/I3 data between different poloxamer stabilisers in the presence of a lipid core or as aqueous solution forming micelles. (C) Fluorescence emission spectra for four different samples of SLNs prepared with for each poloxamer stabiliser (normalised against I1). Shows the difference in I1/I3 values for each poloxamer stabiliser, emphasising the importance of the lipid core from key differences in the excimer emission (440-500 nm).

The *e/m* ratio is a secondary feature of the excimer emission where the height of the excimer peak (~465-470 nm) is divided by the polarity independent peak, I_1. Previous experiments have showed that *e/m* ratios are correlated to the extent of spatial proximity and flexibility of pyrene molecules.[39] A larger *e/m* ratio and excimer emission is due to increased intermolecular coupling of excited pyrene molecules that are more spatially proximal.[39] On the contrary, Pluronic F68 and L64 SLNs both exhibit lower intensity excimer emissions and *e/m* ratios. As previously outlined, our findings suggest adsorption of the PPO block onto the lipid core due to the PEO chains having negligible impact on the internal microenvironment. Therefore, the higher excimer emission and *e/m* ratios for F127 and P105 suggests that the hydrophobic guest molecules are located towards the surface of the lipid core rather than homogeneously distributed throughout the lipid matrix. These findings, coupled with the I_1/I_3 polarity differences, propose that pyrene is more densely packed in SLNs with a less polar lipid core microenvironments and spatial entrapment of guest molecules is largely influenced through the poloxamer PPO block length. These findings are of significant importance as this shows that the choice of poloxamer stabilisers used can profoundly impact the internal polarity of SLNs, without causing a significant difference in the size and PdI of the dispersions. More interestingly, this also introduces a new concept of lipid core polarity tuning which has potential to aid drug loading in future formulations.

Blends of poloxamers are commonly used in formulating SLNs and therefore the effect of blending poloxamers of different PPO block length on the internal core microenvironment was investigated (for blending details see ESI Table S6). No notable difference in the size or PdI of the resulting SLNs for the blends were observed (ESI Figure S7).

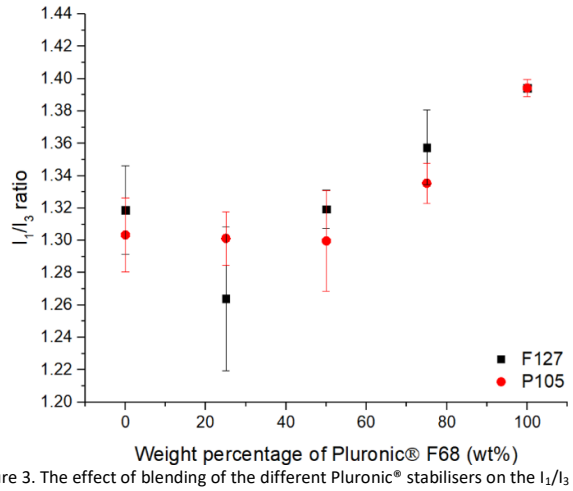

Figure 3. The effect of blending of the different Pluronic® stabilisers on the I_1/I_3 ratio for pyrene loaded SLNs. This shows the effect of varying of the composition of F68 blends with either F127 or P105, showing that increasing the F68 content increases the polarity inside the SLN when using over 50 wt%.

When F127 and P105 were blended (ESI, Figure S8), it was evident that there was a negligible difference in the polarity environment for the SLNs which can be accredited to their similar number of PPO units (65 and 56 respectively). When F68, the poloxamer with shortest PPO block (29), was blended with either of the poloxamer with longer PPO blocks (F127 (65) or P105 (56)), substantial changes in the polarity of the lipid cores was found (Figure 3). With an increase in the amount of F68 in the blend (PPO=29) in both cases caused a notable increase in the polarity of the internal lipid core demonstrating tuneability on Compritol 888 ATO cores and should be taken into consideration during formulation development of drug loaded systems.

In this study we have shown that pyrene can be used as a fluorescent probe to investigate the polarity inside SLNs. We have found that the length of the PPO block in the stabiliser has a significant impact on the polarity inside the lipid core, with this having a larger impact than the HLB of the stabilisers. This understanding of the influence of the length of the PPO block on the polarity inside of the lipid core will be important in the design of SLNs. It may offer the potential to tune the internal environment to enhance drug loading and drug release behaviour.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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