

Scalable manufacturing processes for solid lipid nanoparticles

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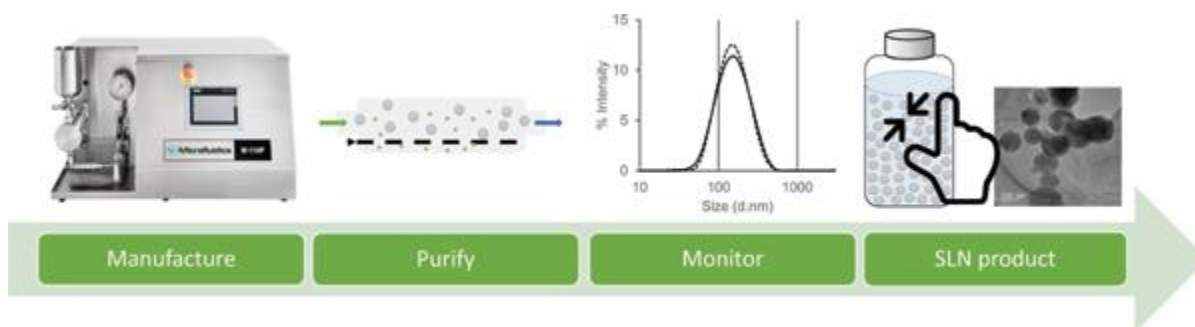
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Abstract

Solid lipid nanoparticles offer a range of advantages as delivery systems, but they are limited by effective manufacturing processes. Within this study we outline a high-throughput and scalable manufacturing process for solid lipid nanoparticles. The solid lipid nanoparticles were formulated from a combination of Tristearin and 1,2-Distearoyl-phosphatidylethanolamine-methyl-polyethyleneglycol conjugate-2000 and manufactured using the M-110P Microfluidizer[®] processor (Microfluidics Inc, Westwood, Massachusetts, US). The manufacturing process was optimized in terms of the number of process cycles (1 to 5) and of process pressure change (20,000, 25,000 and 30,000 psi). The solid lipid nanoparticles were purified using tangential flow filtration, and they were characterized in terms of their size, PDI, Z-potential and protein loading. At-line particle size monitoring was also incorporated within the process train. Our results demonstrate that solid lipid nanoparticles can be effectively manufactured using this process at pressures of 20,000 psi with as little as 2 process passes, with purification and removal of non-entrapped protein achieved after 12 diafiltration cycles. Furthermore, the size could be effectively monitored at-line to allow rapid process control monitoring or product validation. Using this method, protein loaded solid lipid nanoparticles containing a low (1%) and high (16%) Pegylation were manufactured, purified and monitored for particle size using an AT-line system demonstrating a scalable process for the manufacture of these nanoparticles.

Key Words: Solid lipid nanoparticles, Microfluidizer processor, High-throughput manufacturing, protein delivery

GRAPHICAL ABSTRACT



1. Introduction

In the development of nanomedicines, there is a wide range of delivery systems available including polymer based systems, protein based systems and lipid based systems. Within the lipid area, liposomes, emulsions and solid lipid nanoparticles can all be formulated to delivery drugs in a controlled and/or targeting mechanism, with each system offering advantages depending on the drug to be delivered, the rate of delivery, the route of administration and the target site. The use of solid lipids to develop solid lipid nanoparticles (SLNs) offers the potential for longer controlled release compared to emulsions and liposomes, because the drug mobility in these systems is reduced. Solid lipid nanoparticles consist of solid hydrophobic core with a monolayer coating of phospholipid. The solid core contains drug dissolved or dispersed in the solid high melting lipid matrix with the hydrophobic chains of phospholipids also embedded in this matrix ⁽¹⁾. Due to their lipidic nature, solid lipid nanoparticles are biocompatible and they can be manufactured without the use of organic solvents, which is also advantageous. ⁽²⁾ However, solid lipid nanoparticles have limitations including lack of scale-up manufacturing processes and poor drug loading capacity. ⁽³⁾

Currently, there are several formulations on the market based on lipids including a range of liposomal formulations (e.g. Doxil, Ambisome, Myocet) and lipid nanoemulsions such as Diazemuls, Diazepam-Lipuro and Etomidate-Lipuro ⁽⁴⁾. The advantage of the both the liposomal systems and emulsions is the reduced drug side effects, e.g. off-target toxicity (e.g. Doxil) and pain of injection and inflammation at the injection site (e.g. diazepam). Although these systems have an excellent tolerability, the number of products on the market remains relatively low. This is based on manufacturing complexity and cost, in the case of liposomes, and with emulsions it is the physical instability which can be caused by the incorporated drug. In addition, the registered oils such as soybean oil, medium chain triglycerides and long chain triglycerides show an insufficient solubility for drugs of possible interest to be incorporated into emulsions ⁽⁵⁾. In contrast, solid lipid nanoparticles can offer similar advantages to liposomes and emulsion based systems. However, currently there are no clinically approved solid lipid nanoparticle-based medical products.

In terms of their characteristics, generally, the solid lipid nanoparticle lipid matrix determines the pharmaceutical properties of the particles. Currently, stabilizers such as surfactants, co-surfactants and coating materials are widely employed in solid lipid nanoparticle formulations. Antioxidants, electrolytes, preservatives, viscosity enhancing agents, adhesives, absorption enhancers and other excipients can also be added to improve formulations attributes. For example, a combination of Tristearin and pegylated lipids can be used to formulate solid lipid nanoparticles; Tristearin or glyceryl tristearate is a triglyceride derived from three units of stearic acid, widely used in the pharmaceutical filed for its high biocompatibility and biodegradability and approved for oral or parental administration ⁽⁶⁾. Poly (ethylene glycol)-distearoylphosphatidylethanolamine (PEG-DSPE) is a linear PEG phospholipid with saturated C18 stearyl fatty acid ⁽⁷⁾. Once incorporated in the particle, the PEG shell remains on the surface of the matrix and it can generate a hydrophilic coating which potentially reduces the in vivo clearance of carriers and their opsonisation by plasma proteins. The presence of PEG-DSPE could prolong the body circulation time of the carrier and release drugs at a sustained rate in an optimal range of drug concentrations.

Despite the advantages of solid lipid nanoparticles and the variety of formulations that can be explored, there remains a lack of cost-effective and scalable production methods and this has hampered the development of

solid lipid nanoparticles. Current manufacturing and formulations approaches can be classified into three main groups – high energy approaches, low-energy approaches and methods employing organic solvents⁽⁸⁾ and a range of examples of formulations, their method of manufacture and their drug loading is outlined in Table 1. This includes emulsification, extrusion/homogenisation, solvent evaporation methods and the adoption of supercritical fluids. Depending on the method of manufacture and the drug loaded, drug loading varies widely and production must be optimized for the given formulation. However, with many of these methods, dispersion quality is often compromised by the presence of micro particles.

To overcome these technical issues, we have investigated the application of high shear mixing as an innovative manufacture approach to produce protein (Ovalbumin; OVA) loaded solid lipid nanoparticles. One of the key features of high shear processing is the combination of high pressure delivery (which converts into kinetic energy) of the feed-stock into the interaction Y-shaped chamber⁽²⁴⁻²⁶⁾. The Y shape of the chamber separates the stream in two micro-channels. The product stream collides upon itself, producing impaction and shear which reduces the particle size and polydispersity, and by controlling the process parameters (pressure and number of cycles) particle size can be controlled.⁽²⁷⁻²⁸⁾ An additional advantage of this system is the direct scalability of the process from bench to manufacture. Indeed, many papers reported the application of high shear processing for drug carriers manufacturing, especially emulsions, polymer particles, crystalloid solids and liposomes.⁽²⁹⁻³³⁾ For example, soy lecithin based liposomes were demonstrated to have a diameter below 50 nm and low polydispersity after the homogenization process.⁽³⁴⁻³⁵⁾ Furthermore, the Microfluidics M110P was used for particles size reduction of three different liposome formulations composed of 1) hydrogenated soy phosphatidylcholine, 1,2-dioleoyl-3-trimethylammonium-propane and cholesterol, 2) 1,2-dimyristoyl-sn-glycero-3-phosphocholine, 1,2-dimyristoyl-3-trimethylammonium-propane (DMTAP) and cholesterol, or 3) egg phosphatidylcholine, DMTAP and cholesterol. All three of these liposome formulations were shown to be monodisperse and highly stable, with diameter reduced to sub-micron sizes after 3 passages through the Microfluidizer.⁽³⁶⁾ Similarly, this technology has been employed for silica nanoparticle manufacturing and for particles deagglomerating study, with encouraging results for both applications⁽³⁷⁾. Given the positive results with other particulate systems, the aim of this work was to investigate and develop a scalable manufacturing process for solid lipid nanoparticles using the Microfluidizer technology.

2. Materials and Methods

2.1 Materials

Tristearin (Grade II-S, ≥90%) and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich Company Ltd, Poole, UK. 1,2-Distearoyl-phosphatidylethanolamine-methyl-polyethyleneglycol conjugate-2000 (DSPE-mPEG-2000) was obtained from Lipoid GmbH (Ludwigshafen Germany). Ethanol and methanol were obtained from Fisher 141 Scientific UK, Loughborough, UK. TRIS Ultra-Pure was obtained from ICN 142 Biomedicals, Inc., Aurora, Ohio. Phosphate-buffered saline (PBS) and Albumin from chicken egg (OVA), were obtained from Sigma-Aldrich Company Ltd, Poole, UK. Sephadex G-75 size exclusion columns were obtained from GE Healthcare Life Science - Little Chalfont-Buckinghamshire, UK.

2.2 Preparation of solid lipid nanoparticles

Solid lipid nanoparticles were prepared using the M-110P Microfluidizer[®] (Microfluidics Inc, Westwood, Massachusetts, US). mPEG-DSPE-2000 (1 or 16% w/w) was dissolved in 10 mM TRIS buffer at pH 7.4 and heated

up at 75°C. Tristearin 1.3 mg/mL was melted at 75°C and the liquid mass was emulsified with the aqueous phase under vigorous stirring. Buffer solution (75°C) was added to the O/W emulsion to a final volume of 75 mL. The emulsion was loaded in the inlet reservoir of an M-110P Microfluidizer processor and the effect of the number of process cycles (1 to 5) and of process pressure change (20,000, 25,000 and 30,000 psi) were investigated. The size, PDI and Z-potential were measured with Zetasizer Nano ZS and Zetasizer AT (Malvern Instruments Ltd).

OVA loaded Tristearin: mPEG-DSPE (5:1 w/w) solid lipid nanoparticles were similarly prepared: mPEG-DSPE-2000 16% w/w was dissolved in 10 mM TRIS buffer at pH 7.4 and heated up at 75°C. To formulate loaded particles, Ovalbumin (0.1, 0.5 and 1 mg/mL; OVA) was added to the aqueous phase. Tristearin 1.3 mg/mL was melted at 75°C and the liquid mass was emulsified with the aqueous phase under vigorous stirring. Buffer solution (75°C) was added to the O/W emulsion to a final volume of 75 mL. The emulsion was loaded in the inlet reservoir of an M-110P Microfluidizer processor to reduce particles size.

2.3 Purification and concentration of solid lipid nanoparticles using Tangential Flow Filtration

Tangential Flow Filtration (KR2i TFF System®; column cut off 750 kD) as a means of purification and concentration of samples after microfluidizer homogenization. The concentration process after high-shear mixing was performed with a 75-ml feed volume of solid lipid nanoparticles dispersion (0.5 mg/mL) and the TMP maintained at 10 psi. The retentate was circulated back to the feed bottle at filtration speed of 36 mL/min. As more filtrate was generated, the concentration of retentate increased. Once the retentate volume reached 1 mL, purification was stopped. The purified sample was assessed for particle size distribution and zeta potential. Between purification of batches, the TFF system was cleaned by 1-h continuous circulation of 0.1 M NaOH followed by flushing with a large volume of water (≥ 1 L).

2.4 Characterisation of solid lipid nanoparticles

2.4.1 Characterisation of particle size and zeta potential.

The dynamic light scattering (DLS) technique was used to report the intensity mean diameter (z-average) and the polydispersity of all solid lipid nanoparticles formulations (Malvern Zetasizer Nano-ZS (Malvern Instruments, Worcs., UK)). The measurements of particles size and polydispersity were carried out at 25 °C in Tris buffer (1:10 dilution, 10 mM, pH 7.4). Liposome zeta potential was measured in Tris buffer (1:10 dilution, 10 mM, pH 7.4) using the Malvern Zetasizer Nano-ZS (Malvern Instruments, Worcs., UK). All measurements were undertaken in triplicates. Furthermore, The Zetasizer AT (Malvern Instruments, Worcs., UK) was used as an on-line measurement of particle size. 2.5 mL of samples were diluted 1:10 in Tris buffer (1:10 dilution, 10 mM, pH 7.4) at 25 °C and the volume was let circulating between the mixing tank and the homogenizer, until the measurement was completed. Data obtained with the two instruments were compared.

2.4.2 Lipid recovery

Lipid recovery has been performed adding 1,1'-Dioctadecyl-3,3',3'-Tetramethylindocarbocyanine Perchlorate (DiIC) 0.2% mol total lipid concentration, solved in the melt lipid before being loaded in the Microfluidizer. DiIC sample fluorescence was measured before and after TFF (PolarStar, BMG LABTECH GmbH). Lipid quantification was achieved by referring to a calibration curve with a linearity of $R^2=0.995$. All measurements were within the level of detection and level of quantification.

2.4.3 Protein loading quantification

The loading efficiency was measured using reverse phase HPLC (Agilent 1100 Series). The experiment was performed with a mobile phase of TFA 0.1 % and Methanol with 0.08% TFA with a flow rate of 2.0 mL/min, λ_{max} of 215 nm. At these conditions the OVA retention time is 9.6 min. The particles were destructed using a solution of IPA: TRIS 50:50 vol/vol. The solution was left at room temperature for 1 hour to achieve the complete particle dissolution. Then 1 mL of that solution was used for HPLC. The protein concentration was measured by reference to a calibration curve with a linearity of $R^2 = 0.999$. All measurements were within the level of detection and level of quantification.

2.4 *In vitro* release of protein from solid lipid nanoparticles

For the release study of ovalbumin from solid lipid nanoparticles, nanoparticles were prepared using Microfluidizer (number of passes 5, pressure 25000 psi). All formulations were dialysed against 80 mL PBS pH 7.4 at 37 °C (membrane cut off 300 KDa). The absorbance of aliquots from the outer buffer was analysed at different time points (up to 48 h) using NanoDrop 2000c UV-Vis spectrophotometer (Thermo Fisher Scientific Inc). The amount of released OVA was detected by measuring the protein absorbance at 230 nm (NanoDrop 2000c, UV-Vis Spectrophotometer) and related these values to the concentration using a calibration curve (linearity $R^2 = 0.998$).

2.5 Statistical analysis

Unless stated otherwise, the results were calculated as mean \pm standard deviation (SD). ANOVA followed by Tukey post hoc analysis was performed for comparison and significance was acknowledged for p values less than 0.05. All the calculations were made using Excel (Excel Software and Systems Pvt. Ltd.).

3. Results

3.1 High-throughput manufacturing of solid lipid nanoparticles via high shear mixing

Solid lipid nanoparticles consisting of Tristearin and mPEG-DSPE-2000 were formulated using High Shear Mixing method. The aim was to optimise parameters to control particle size by varying the operating pressure from 20,000 to 30,000 psi as well as the number of circulation cycles from 1 to 5. Figure 1A demonstrates that all particles produced by Microfluidizer processor had a diameter of 250 nm or below with a polydispersity index between 0.2 and 0.3. The results also show that changes in pressure values between 20,000 and 30,000 psi did not significantly influence particles size; however, at pressures of 20,000 psi the variability within the particle size was the lowest (Figure 1A) therefore demonstrating that this pressure can be adopted to produce solid lipid nanoparticles. Furthermore, increasing the number of cycles did not significantly reduce the particle size and at 20,000 psi particles of around 200 nm and polydispersity around 0.25 were formed irrespective of the number of passes demonstrating the particles are easily formed via this method. When considering the zeta potential of the formulations, all solid lipid nanoparticles were anionic in nature, as would be expected given their composition (approximately -20 to -30 mV; Figure 1B) and these values were not significantly affected by either number of cycles or applied pressure.

Table 1. Examples of solid lipid nanoparticles formulations and their method of manufacture.

Manufacturing method	Formulation	Drug	LE%	Ref.
double emulsion method (w/o/w) and solvent evaporation	Lecithin and triglyceride	Catalase	50	9
	Phosphatidylcholine and tripalmitin	Catalase	78	10
double emulsion and melt dispersion	stearic acid or a mixture of stearic acid and crodamol	Sulforhodamine 101	60	11
high pressure homogenisation cold dispersion	Witepsol E 85, Softisan 142, propyleneglycol, Superpolystate, cetyl alcohol	Lysozyme	40	12
	stearic acid	Cyclosporine	70-90	13
high pressure homogenisation hot dispersion	stearic acid	Cyclosporine	80	14
Warm microemulsion	Egg lecithin, stearic acid	Thymopentin	<5	15
	mixture of stearic acid and Epikuron 200®,	Cyclosporine	13	16
	stearic acid and lecithin	Insulin	38	17
supercritical fluid	Gelucire® 50-02 and Dynasan® 114	bovine serum albumin	13	18
	Phosphatidylcholine and tristearin	insulin and recombinant	<3	19

		human growth hormone		
	tristearin/phosphatidylcholine/dioctyl sulfosuccinate	Insulin	<3	20
Solvent displacement	Monostearin	Gonadorelin	50	21
	Lecithin	Insulin	27	22
Solvent evaporation	glyceryl tripalmitate	Thymocartin	<50	23

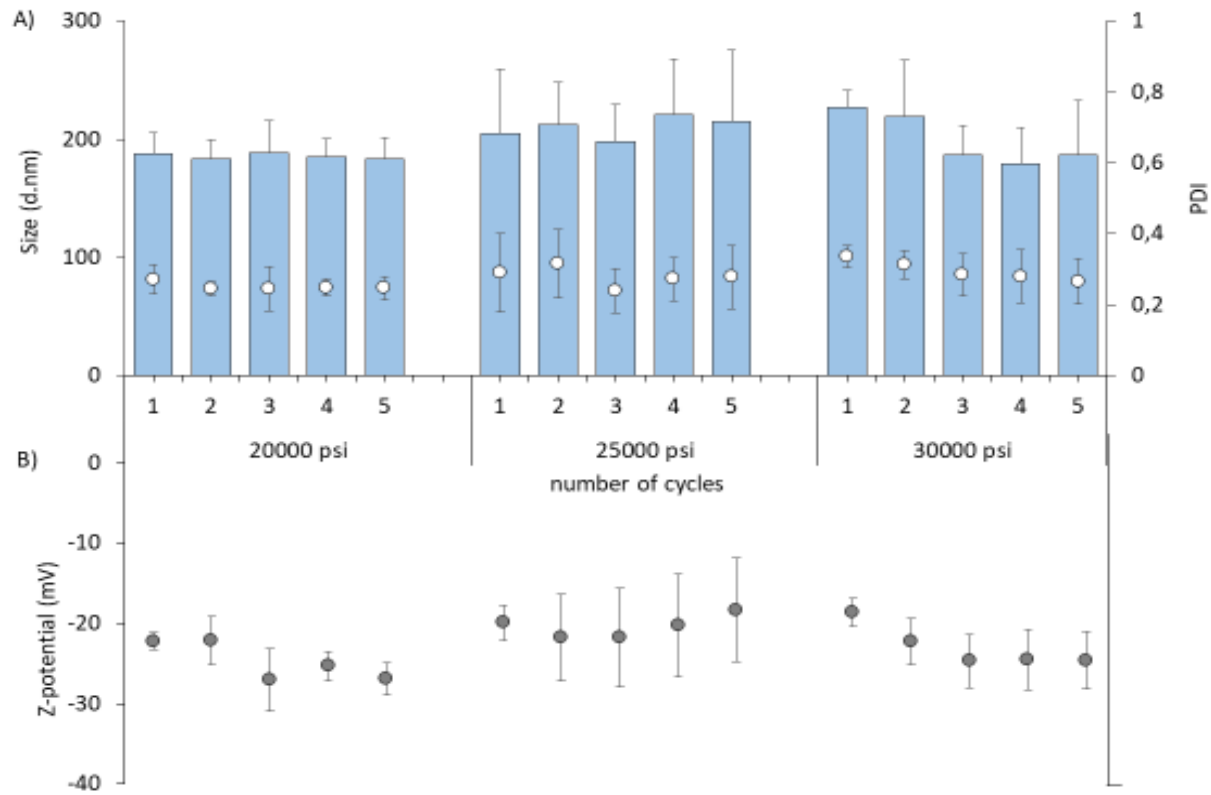


Figure 1. The effect of processing pressure and passes on SLN attributes. A) Size (columns), polydispersity (dots) and B) Z-potential of Tristearin:mPEG-DSPE SLNs obtained with Microfluidizer processor. Sample with pressure from 20000 to 30000 psi as well as cycles number from 1 to 5 had been tested. Results are expressed as the means of three experiments \pm S.D.

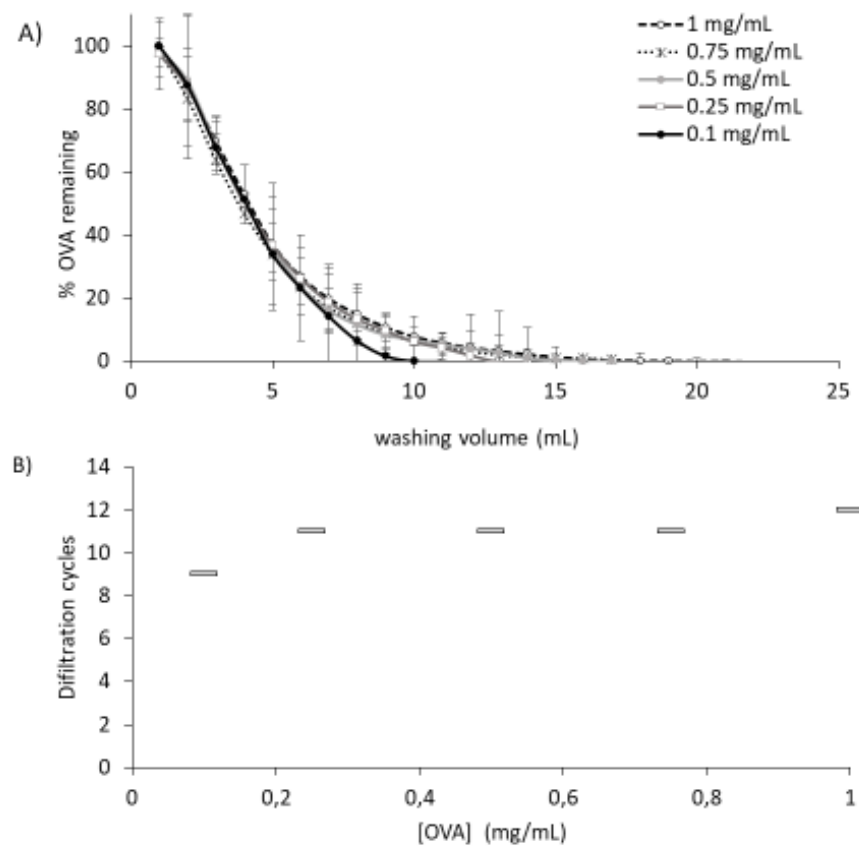


Figure 2. Purification and removal of non-incorporated protein via TFF. A) Percentage of remained OVA (mg/mL) after 20 washes at initial protein concentrations from 0.1 to 1 mg/mL. B) Minimum number of difiltration cycles required to have a protein remained concentration below 5% (w/w). Results are expressed as the means of three experiments \pm S.D.

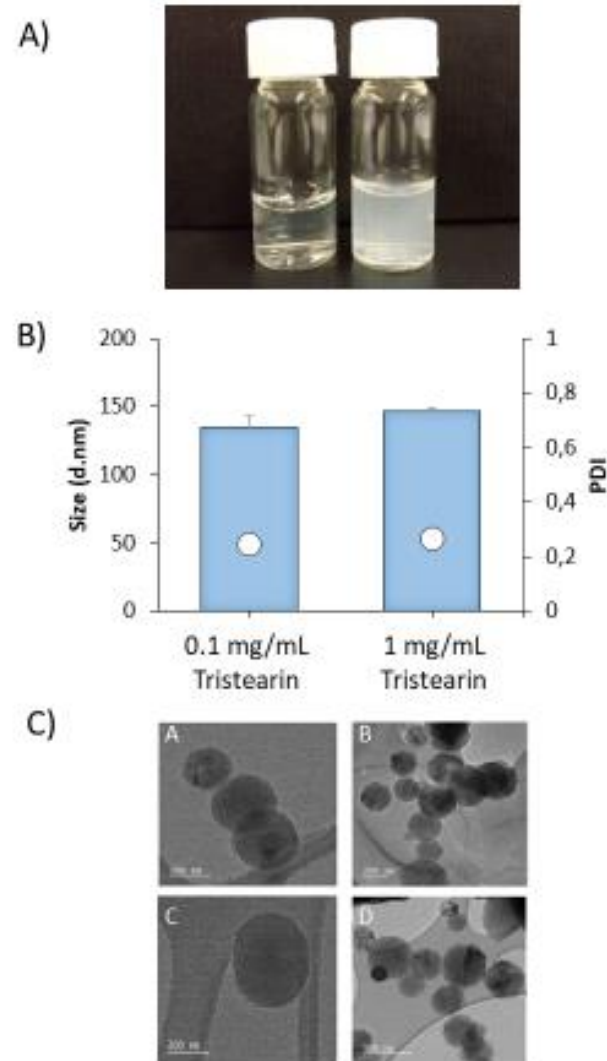


Figure 3. Production and at-line monitoring of SLNs after production via Microfluidizer processor after concentration via TFF in terms of A) visual appearance, B) Size (columns) and PDI (dots) of Tristearin: PEG SLNs before and after 10 times TFF concentration and C) cryoTEM images of Tristearin: PEG SLNs made by Microfluidizer processor before (on the left) and after (on the right) 10 times TFF concentration. Where appropriate, results are expressed as the means of three experiments \pm S.D.

3.2 Development of a high-throughput purification of solid lipid nanoparticles and in-line particle size monitoring

To support the rapid purification and concentration of solid lipid nanoparticles, we applied tangential flow filtration (TFF). Initially, the TFF process was optimized to ensure effective removal of non-incorporated protein with protein concentrations of 0.1 to 1 mg/mL being mixed with pre-formed solid lipid nanoparticles and the removal of Ovalbumin measured. The results in Figure 2A demonstrate that across this range the purification process was similar with 12 difiltration cycles effectively removing non-loaded protein (Figure 2B). This process was then used to purify solid lipid nanoparticles prepared with OVA loaded. The lipid recovery and the SLN attributes (size, pdi, zp and protein loading) was measured for Tristearin: PEG SLNs (5:1 w/w) after purification via this TFF protocol (Table 2) with the SLNs being approximately 140 nm in size with a PDI of 0.2 with high protein loading (100 µg/mL) and high particle recovery was 96% (Table 2). The SLNs were also subjected to a 50 times concentration step via TFF, and there was no significant change in particle size and PDI (Figure 3B) or morphology (Figure 3C).

An important parameter in the development of effective manufacturing is the ability to rapidly monitor critical quality attributes of a product and in the case of nanoparticles, particle size is a key attribute. Therefore, we investigated the potential to monitor the particle size of our SLNs using the Zetasizer AT which offers on-line measurement of particle size using Dynamic Light Scattering to enable read time monitoring of the particle production. To achieve this, we set up the system at-line, such that samples could be tested during production as a process monitoring control or alternatively at the end of the production as a quality control. We compared this to the standard off-line particle sizing (Malvern Zetasizer). Figure 4 outlines the set-up and demonstrates that continuous monitoring of the particles size and polydispersity after TFF purification and concentration was achieved with no significant difference in particle size when measure off-line and at-line.

3.3 Manufacture of protein-loaded solid lipid nanoparticles

To assess the ability of the M110-P Microfluidizer processor as a potential high throughput manufacturing method for protein loaded lipid particles production, we formulated Tristearin:PEG SLNs in presence of different protein (Ovalbumin -OVA) concentrations. As it shown in figure 5A increasing the protein concentration had an impact on particles attributes with higher protein concentrations increasing the particle size with high variability 250 ± 100 nm in size and a PDI of 0.4 ± 0.05 . However initial OVA concentrations of up to 0.5 mg/mL has no significant impact on particle size (184 ± 31 nm and a PDI of 0.36 ± 0.03) similar to particles formulated with initial OVA concentrations of 0.1 mg/mL OVA concentration (Figure 5A). The initial protein concentration did not impact on zeta potential with all particles being around - 30 to - 35 mV (Figure 5B) and OVA loaded within the SLNs could be increased up to a maximum of 100 µg/mL for the given lipid concentration (Figure 5C).

Finally, we tested the impact of increasing the amount of mPEG within the SLNs given that low levels of PEG may be useful in the formulation of vaccine delivery systems whilst high levels of PEG can be useful for avoiding rapid clearance from the circulation. As shown in Figure 6, increasing the amount of PEG had no significant effect on particle size, zp, protein loading and release profiles, suggesting that these systems can be prepared with low and high degrees of Pegylation without impacting on the particle size, PDI, loading and release properties.

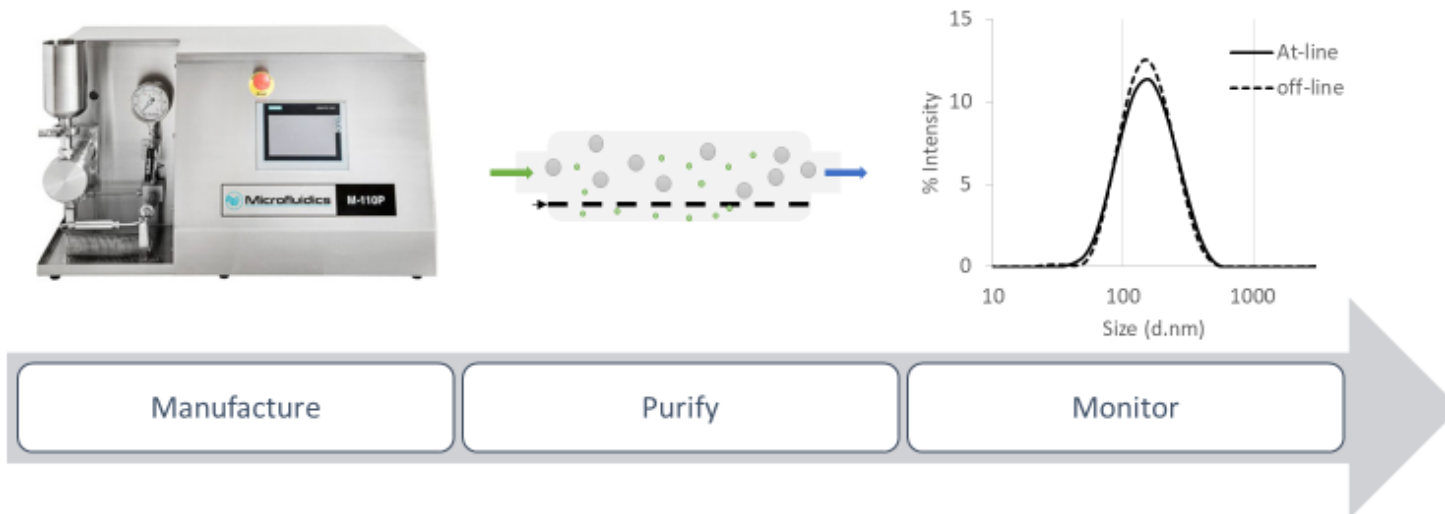


Figure 4. Production and at-line monitoring of SLNs after production via Microfluidizer processor. SLNs were produced, purified via TFF and the particle size measured by circulation between the mixing tank and the homogenizer, until complete detection. Data obtained with at-line and off-line dynamic light scattering were compared.

Table 2. solid lipid nanoparticles attributes after purification via TFF. Results represent mean \pm SD, n = 3.

Solid lipid nanoparticles attributes	
Size	142 \pm 3 nm
PDI	0.23 \pm 0.01
Protein loading capacity	100 \pm 24 μ g/mL
Particle recovery	96 %

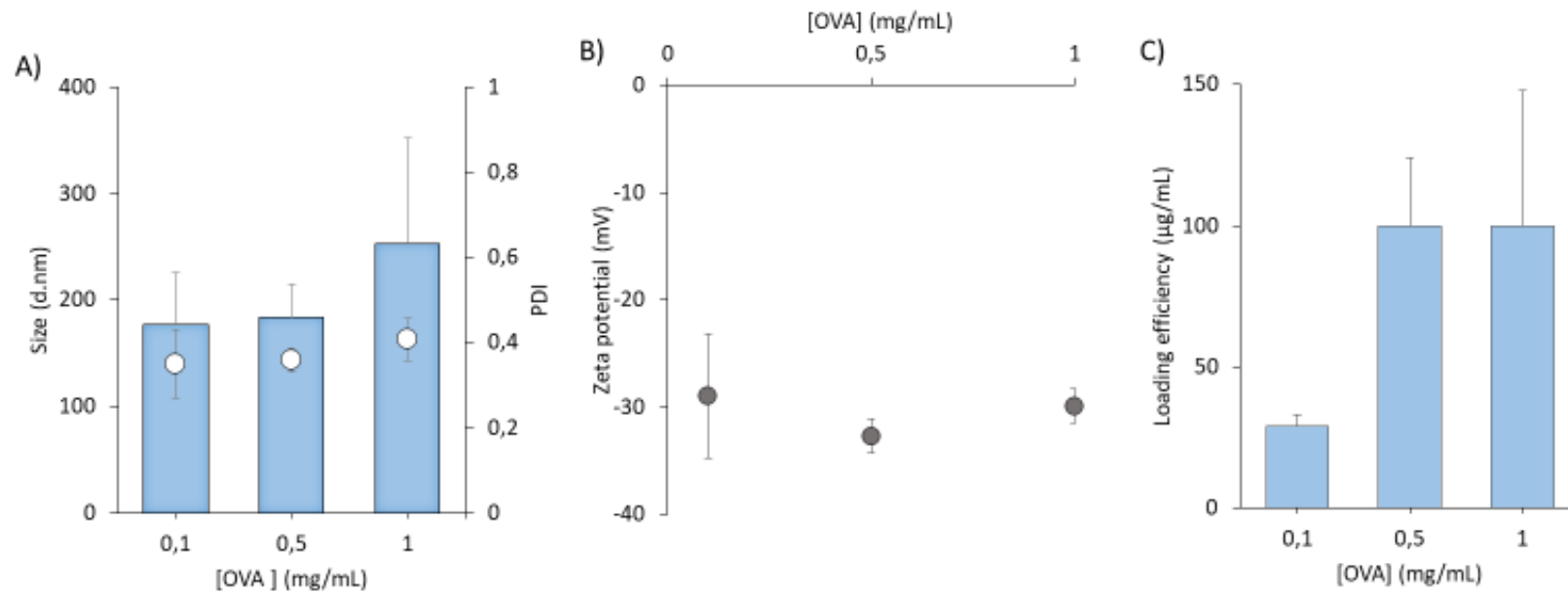


Figure 5. OVA-loaded solid lipid nanoparticles made by Microfluidizer processor a) Size (columns), PDI (dots) and b) Zeta potential and c) Loading efficiency of Tristearin: mPEG-DSPE SLNs expressed as percentage of the initial protein amount ($\mu\text{g/mL}$). Results are expressed as the means of three experiments \pm S.D.

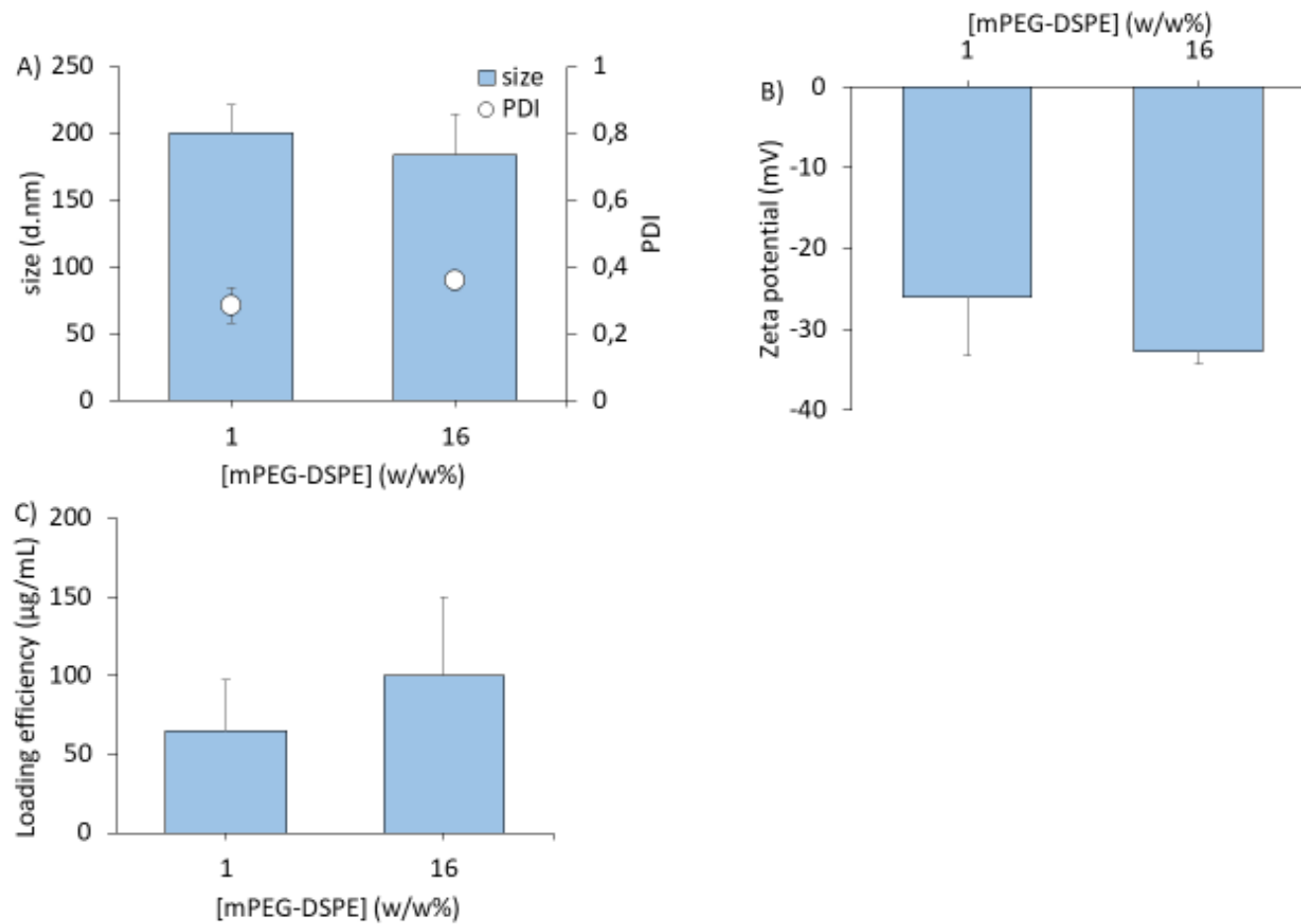


Figure 6. The effect of PEG on SLN attributes A) size and PDI, B) Zeta Potential and C) protein loading. The initial loading protein concentration used to produce the formulation was 0.5 mg/mL. Results are expressed as the means of three independent experiments \pm S.D.

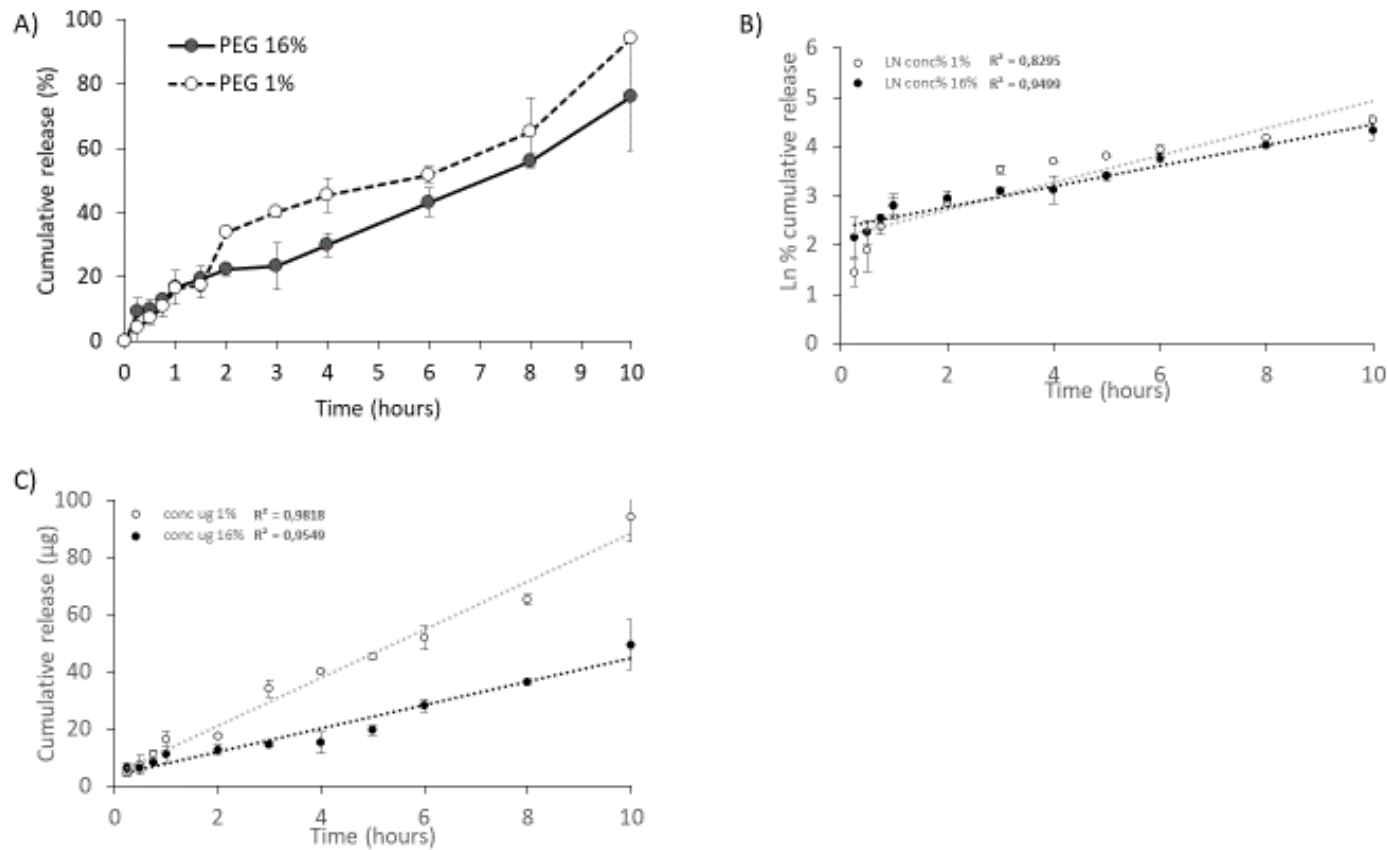


Figure 7 A) The cumulative release profile of OVA under physiological conditions from SLNs (PBS buffer, pH = 7.4, at 37°C) from formulations containing either 1% (open circle) or 16% (black circle) w/w PEG. The initial loading protein concentration used to produce the formulation was 0.5 mg/mL. Results represent percentage cumulative release of initially incorporated OVA. Data was also replotted according to B) zero-order or C) first-order. Results are expressed as the means of three independent experiments \pm SD.

4. Discussion

Solid lipid nanoparticles offer a range of advantages for drug delivery due to their biocompatibility, biodegradability and physical stability. Despite these advantages, their application has been limited by the lack of cost-effective and scalable methods for their preparation (Table 1). Furthermore, the loading of hydrophilic, biologically active compounds is challenging, due to the intrinsic lipophilic nature of solid lipid nanoparticles. High shear mixing is commonly applied this method for particles size reduction with decreasing particles size occurring after a few recirculation cycles⁽³⁸⁻³⁹⁾. For example, liposomes made by 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, 1,2-dipalmitoyl-sn-glycero-3-phosphoglycerol and cholesterol reached size values of around 80 nm and PDI 0.11 after 12 cycles at 10,000 psi⁽⁴⁰⁾. Similarly, liposomes prepared by this method and formulated from hydrogenated soybean phosphatidylcholine and N-(Carbonyl-methoxypolyethyleneglycol2000)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine (MPEG2000-DSPE) were highly homogeneous with average diameter of 90-110 nm⁽⁴¹⁾. Generally, solid lipid nanoparticles tend to be larger in size and previous studies using three recirculating cycles in microfluidizer platform at 10,000 psi produced Tristearin based SLNs at around 200 nm with low polydispersity⁽⁴²⁾ and within our studies (Figure 1) we demonstrate this method can be employed to produce SLNs in a high through-put and efficient production of SLNs at flow rates of up to 155 mL/min depending on the process pressure.

In addition to their productions, solid lipid nanoparticles (and other nanoparticles) generally require purification to remove non-entrapped drug and tangential flow filtration offers a scalable process. Therefore, with has been exploited in our manufacturing process to effectively remove un-entrapped protein with our SLNs being purified and retaining their physico-chemical attributes (Figure 2, 3 and 4) with good particle recovery (Table 2). TFF has recently emerged as an innovative purification method, and several studies describe the effective purification and concentration of nanoparticles using tangential flow filtration⁽⁴³⁻⁴⁴⁾. For instance, nanoparticles composed of poly (D, L-lactide-co-glycolide) and polyvinyl alcohol (PVA) were purified by tangential flow filtration with a 300K MWCO membrane and the purified particle dispersions were stable and free of aggregation⁽⁴⁵⁾. Moreover, it was demonstrated that phosphatidylcholine and cholesterol based liposomes were successfully purified by TFF application, with no change in particles size (approximately 115 nm) and PDI (0.15) and a total lipid recovery after diafiltration⁽⁴⁶⁾. We then took advantage of TFF to remove unbounded protein; Ovalbumin was used as model biomolecule which is proven to be biologically active as immunostimulant⁽⁴⁷⁻⁴⁸⁾, thus it can be applicable in the development of both drug and vaccine delivery formulation assessment. As a water soluble compound, its encapsulation in lipid based systems is challenging. Therefore, the choice of OVA is a good rational to challenge our production method. Herein, we proved that protein loading solid lipid nanoparticles could be manufactured, purified and concentrated using scalable and cost-effective methods, without any alterations in particles physicochemical properties. Within these studies, we also demonstrate that high protein concentrations destabilize the inner colloidal structure of particles, increasing sizes and inducing aggregation.⁽⁵¹⁻⁵³⁾ Many factors can physically destabilize solid lipid nanoparticles leading to significant increase in particles size and consequent aggregation. The use of PEG in particles formulation is a well-known technique to improve physical stability of particles. PEG generates hydrophilic polymeric coating and PEG chains help to keep particles separated.⁽⁵⁴⁻⁵⁶⁾ The presence of PEG shield favours particles repulsion thus reducing particle growth and collapse.

In vitro release study from our SLNs revealed that protein release increased over time with no burst effect (Figure 6); after 10 hours all protein content (>80% of the initial concentration) was released irrespective of the PEG

content. Drug release from almost all the SLNs follows Weibul and Higuchi equations better than first-order equation ⁽⁵⁷⁻⁵⁸⁾. The pseudo-first order release kinetics maintains the drug concentration in the blood or target tissue at a desired value longer ⁽⁵⁹⁾. Furthermore, the presence of high amount of PEG reduced kinetics of the process: several coherent explanations could be given to this phenomenon. According to Stocks-Einstein equation ⁽⁶⁰⁾, diffusion coefficient is inversely proportional to solution viscosity: diffusion could be slowed down by increasing solution viscosity. The presence of polyethylene glycol molecules on particles surface enhanced the bulk viscosity of water reducing the speed at which OVA could cross the lipid barrier. Secondly, PEG chains are very flexible and they give larger hydrodynamic radius in aqueous media. According to Fick's first law of diffusion ⁽⁶¹⁾, the rate of diffusion is related to membrane thickness, in a reverse proportional manner. The presence of polymer increased the thickness of particles surface and reduced OVA diffusion speed. All these findings emphasise the applicability of lipid particles and microfluidizer as a valid alternative for proteins production.

5. Conclusions

The work reported here demonstrates that we can effectively formulate, manufacture, purify and monitor the particle size of SLNs produced in process that scales from bench to clinic. Within this study we have formulated SLNs at approx. 200 nm or below containing low and high PEGylation (1 and 16 mol%) and incorporating OVA. The modification of the PEGylation will allow us to explore these delivery systems as potential adjuvants (low PEGylation) and for therapeutic drug delivery (high PEGylation). We are able to manufacture the SLNs rapidly (2 cycles) and at a low pressure (20,000 psi) and removal of non-entrapped protein achieved after 12 diafiltration cycles. This work outlines a simple and scalable manufacturing process that can enhance the translation of SLNs from laboratory tools to clinical products.

6. Acknowledgements.

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7. Supporting information Available

Data presented in this publication can be found at DOI [10.2174/2211738507666190925112942](https://doi.org/10.2174/2211738507666190925112942)

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