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SLNs can Serve as the New Brachytherapy Seed: Determining Influence of Surfactants on Particle Size of Solid Lipid Microparticles and Development of Hydrophobised Copper Nanoparticles for Potential Insertion

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Abstract

Solid lipid nanoparticles (SLNs) or technically speaking, larger solid lipid microparticles are emerging as a non-invasive technique for drug-delivery, carrying forward advantages of conventional nanostructured systems and eliminating their drawbacks. This study involves the analysis of solid lipid microparticles generated via hot high-shear homogenization technique with tristearin as the main lipid component and phosphate buffer (pH 7.00) as the aqueous phase (phase volume 0.2). This study aims to identify the effects of the chosen lipid, and four non-ionic surfactants (Tween®20, Tween®80, Lutrol F68 and Lutrol F127) on particle size of solid lipid microparticles. Samples generating particle sizes of less than 200 nm were further analysed via SEM to determine morphological characteristics of SLNs. SLN composition is crucial as it determines various chemical and physical properties of the particle itself. Firstly, selection of lipids is key, as it the major component of the particle. Lipids display different polymorphic transitions upon crystallization affecting loading efficiency, drug distribution, drug loading, particle size, particle shape and overall stability. Secondly, selection of surfactants is vital as they overcome stability problems with reducing surface tension, particle aggregation and steric interactions. The aim of this study was to determine the optimal functioning surfactant concentration that would produce particle sizes less than 200 nm. SLNs generated in this study displayed a reduced particle size (90-150 nm) when using 2% w/v Tween®80 plus 5:1 F68; higher molecular weight surfactant, with a low molecular weight poloxamer. Based on previous research, these results can be explained via the surfactant characteristics in solution. The length of the fatty acid chains associated with Tween®20 and 80 provide a potential behavioural pattern on the SLN; longer chains surrounding the SLN structure increase stability of the particle but also increase particle size. Poloxamer use is that of a co-surfactant, fulfilling what Tween® fails to complete. In which higher molecular weight poloxamers can potentially explain the large particle size as the longer PEO terminal chains protrude from the particle after its insertion.

Keywords: Solid lipid nanoparticle; SLN; Particle size; Copper; Tween® 20/80; Lutrol® L68/127

Introduction

Nanotechnology has definitely altered the conventional formulation of medicine as a whole, allowing production of efficient drug delivery systems. The 21st century embarks a new journey for the field of medicine, as the groundwork of research from past years have led to vast improvements in drug-delivery efficacy. The concept of using a nano-approach has opened doors to many drug treatment possibilities, allowing the option to generate non-invasive techniques and proficient drug therapy regimes. It incorporates the use of nano-sized vehicles as drug carriers to improve bioavailability of the drug from various administration routes. The most popular, and recently developed, solid lipid nanoparticles (SLNs) are the new pharmaceutical entities that have emerged as a novel form of drug-delivery system. They possess several advantages over many of the conventional colloidal carriers and colloidal systems, such as emulsions, liposomes and polymeric nanoparticles.

Solid lipid nanoparticles are spherical in shape and range from a particle size diameter of 10-1000 nm. Many scientists use the term nano to refer to sizes of less than 100 nm. They consist of a biocompatible solid lipophillic matrix, covered by a phospholipid monolayer coating [1,2]. Drug particles can either be dissolved or dispersed in the lipid matrix or concentrated in the inner and/or outer shell, providing different drug-release kinetic profiles (Figure 1). In addition, drug particles are also adsorbed or attached onto the surface of the SLNs, behaving similar to that of a PEGylated liposome.

Solid lipid nanoparticles are prepared from lipids which are solid at room temperature such as triglycerides, glyceride mixtures, waxes, hard fats with the incorporation of surfactants [3,4]. They can be formulated using various different techniques; High-shear pressure homogenization (Hot or Cold), Ultrasonication, Microemulsion-based SLN preparation, Double emulsion etc. [5-7]. This research will outline the use of hot high-shear pressure homogenization for formulating SLNs.

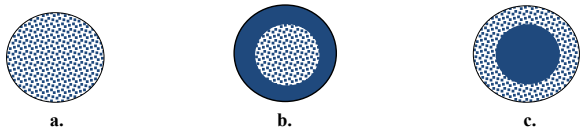

Figure 1: Three models of drug-incorporation into a solid lipid nanoparticle [3,5]. Homogeneous matrix model, drug-enriched core model, drug-enriched shell model, respectively.

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As opposed to the conventional liquid-lipid systems, the solid nature of the SLN provides an improved physical and chemical stability profile of the carrier system [1]. This feature enables efficient drug entrapment in the SLN, preventing drug leakage from the system and also providing protection of the drug from chemical degradation prior and post ingestion. This enhances efficacy, promoting increased bioavailability of the drug, thus improving treatment profile. By using biocompatible and biodegradable substances to formulate SLNs, they increase patient safety as they contain significantly low toxicity *in vivo*. The solid structure of the lipid matrix allow for more improved and sustained drug release mechanisms due to the zero-order kinetic breakdown of the SLN [4,6]. SLNs allow the ability to incorporate various drug moieties, both lipophillic and hydrophilic, combating disadvantages of many drug delivery designs, with a relative cost-effective approach for large scale production. In contrast, SLNs also display a few disadvantages but at a small scale. Firstly, SLNs experience poor drug loading capacity, especially for hydrophilic molecules due to partitioning effects [6,8]. They contain a higher water content in their dispersions of between 70-99.9% [4-6]. Also, drug expulsion occurs during storage as a result of polymeric phase transition [8].

Juxtaposing the advantages with the nanometer size range, SLNs are now being tested for cytotoxic research for cancer studies. The highly vascular nature of cancerous tissue force treatment regimes to be aggressive and vigorous in nature, generating unwanted serious side effects. The issue lies in the insensitivity and lack of specificity of the drug vehicle affecting both healthy and non-healthy cells. The severity of the side-effects cause decreased tolerability resulting in poor patient outcome. The increasing bioavailability of the drug at the action site by SLNs significantly reduces systemic drug toxicity, and in turn, promotes higher concentration of drug levels in the affected tissue [1,5,7,9]. Particles with sizes greater than 10 μm are easily susceptible to phagocytic uptake by macrophages and RES (reticuloendothelial system), therefore an optimal particle size is required that would retain in the tissue and not be removed. The nanometre size range of these particles persist well in the leaky vascular nature of the tumour [9,10]. Hence, formulating SLNs to fit the range of 5.5-200 nm, would allow easy access to tissue and avoid clearance mechanisms, retaining drug vehicle at the target site [11]. This not only enhances drug targeting but also exploits potency of the drug itself.

This research involves a miniature version production scale of SLNs, prepared by hot high-shear pressure homogenization, to observe effects of surfactants (Tween®20, Tween®80, Lutrol F68 and Lutrol F127) and homogenization time on particle size.

As a side note, an external experiment was conducted as a means of testing metal incorporation into a SLN, potentially functioning as a brachytherapy 'seed'. The aim of this study was to develop a coating technique for a metal entity representing a radioisotope, and enable its partitioning into an organic phase, thus assuming a successful incorporation into the highly lipophillic SLN. Copper (II) nanopowder was used as a mimic for radioisotopes and tested for partitioning capabilities post-coating with propionic acid.

Materials and Methods

Materials

Glyceryl tristearate (Tristearin; Lot: BCBJ3956V), Tween®80 (Lot: BCBL5104V), Tween®20 (Lot: SZBDV2190V), and copper nanopowder (40-60 nm particle size SAXS; 99.9% trace metal basis, Lot: MKBN8930V) were purchased from Sigma-Aldrich Corporation. Copper solution (1000 ppm in ca. M nitric acid copper (II) ion single element solution, Lot: 1374195) and diethyl ether (laboratory reagent grade, Lot: 1344538) were purchased from Fisher Chemical. Lutrol® F127 NF M (polyoxypropylene-polyoxyethylene block copolymer; Lot: WPDW534CV5) and Lutrol® F68 NF M (polyoxypropylene-polyoxyethylene block copolymer; Lot: WP1V589BV2) were purchased from BASF Corporation. Propionic acid (99% pure; Lot: A0326123) were purchased from ACROS Organics. $Na_2HPO_4{\cdot}2H_2O$ and $Na_2HPO_4{\cdot}2H_2O$ were provided by the University of Brighton laboratory, to formulate phosphate buffer calibrated at pH 7.00. These chemicals were used as per given without any further purification modifications. All samples were prepared by weight.

Essential cleaning of glassware and other laboratory materials

All glassware, magnetic stirrers and other materials which were to come into contact with formulation ingredients used, were carefully washed using chromic acid and rinsed with purified water (PureLab Flex, 18.2 MΩ). All materials intended to be used were rinsed and left to air dry prior to usage.

Part I: Solid lipid nanoparticles

Preparation of Solid lipid nanoparticles (SLNs): SLNs (solid lipid microparticles) were prepared using hot high-shear homogenization technique with tristearin as the main lipid, and surfactants (Tween®20/Tween®80 and Lutrol® F127 NF M/ Lutrol® F68 NF M). It should be mentioned that Tween® series were added in different concentrations, separately, (10%, 5%, 3%, 2% w/v) to the emulsion mixture and the Lutrol series were maintained at the 5:1 poloaxmer ratio with each Tween® at each separate Tween® concentration. Phase volume ratio of 0.2 was used as the basis for hot o/w emulsion (80% aqueous phase, 20% lipid phase). Phosphate buffer calibrated at pH 7.00 was chosen as the aqueous phase. The quantities of Tween®20 and Tween®80 was determined based on the total volume of the mixture with the designated concentrations in each mixture from which separate batches of mixtures were made (Table 1).

The aqueous medium was placed on a magnetic stirrer hotplate and heated to 75-80°C to ensure complete melting of the lipid (tristearin) upon addition. Once acquired temperature was established, tristearin was added to the aqueous phase under magnetic high speed stirring

Batch(s)*	Surfactant (Tween® series)	Concentration of surfactant (w/v)	Poloxamer (ratio 5:1)
1	Tween®20	10%	L68
2			L127
3		5%	L68
4			L127
5		3%	L68
6			L127
7		2%	L68
8			L127
9	Tween®80	10%	L68
10			L127
11		5%	L68
12			L127
13		3%	L68
14			L127
15		2%	L68
16			L127

Table 1: Composition of batches with phase volume of 0.2 tristearin.

(800 RPM) to avoid lipid phase separation, forming a successful o/w emulsion. Following complete melting of lipid, combinations of Tween® and Lutrol were added in their appropriate constituted amounts forming samples 1-16. Upon addition of surfactants, the pre-emulsion was kept under high speed stirring for 15 minutes followed by high-speed homogenization at 8000 RPM (Homogenizer Polytron PY 10-35 GT-D; Made in Switzerland by Kinematica AG). The emulsion temperature was maintained throughout the entire process between 75-80°C, aiming to keep lipid above its melting point at all times during the homogenization process. Samples of hot emulsion (~20 mL) were extracted using a glass pipette at time intervals of 5, 10, 20, 30 and 40 minutes during the homogenization cycle, and transferred into small transparent glass sample tubes. These were allowed to cool at room temperature 24°C for 1 hr and then stored at 6-8°C prior to analysis.

Determination of particle size: Particle size and size distribution of SLNs was measured using Malvern Zetasizer Nanoseries ZS90 H214a (Malvern Instruments, Worcestershire, UK). Particle size is measured via dynamic light scattering that quantifies the fluctuation of scattered light intensity from the movement of particles. Samples were filtered using 0.45 µm filters (PALL GxFNylon, Zymark Automation Certified). All sample batches were diluted 1:10 using fresh phosphate buffer (pH 7.00) prior to analysis, and added in 10% ethanol/water rinsed disposable cuvettes. Measurements were taken as single and replicates of two, with 30 runs per sample (5 second interval between runs) at 25°C. Particle sizes of all samples were measured in terms of intensity (%); particular interest in intensities greater than 50-55%. Samples that generated particle sizes less than 200 nm were considered for further analysis.

Scanning electron microscope (SEM): size analysis: A SEM analysis was conducted to observe morphological characteristics of selected solid lipid microparticles. Prior to analysis, approximately 1 mL of each sample was placed on a double-sided carbon tape mounted onto an aluminium stud, and dried in a desiccator for 48 hrs. SEM images were recorded on a Jeol, JSM 5310, (Tokyo, Japan) scanning electron microscope, with an acceleration voltage of 25 kV.

Part II: Copper nanoparticles

Preparation of hydrophobised copper nanoparticles: Copper nanopowder (40-60 nm particle size) purchased from Sigma-Aldrich was used with propionic acid (99% pure; ACROS Organics) as the derivatisation component. Firstly, two different solutions of propionic acid were made using 50 mL of pure water (0.1M and 1M) in 100 mL volumetric flasks. In each solution, 0.05 g of copper nanopowder was added, in aims of forming an acid-metal complex. After performing serial dilutions for each solution, a final concentration of 0.398 ppm of copper (assumed to be in an acid-metal complex) was present in 25 mL of pure water. These samples were labeled A, B; 0.1M and 1M respectively. Secondly, 25 mL of diethyl ether (laboratory reagent grade) was used as the organic solvent and added to each sample. Samples A and B were manually stirred using a glass stirring rod to enable phase interaction. Samples were set stable, untouched for 30 minutes prior to phase extraction.

Liquid-liquid extraction of separate phases: Both samples were poured into different separating funnels. Samples were collected into a 50 mL round-bottom flask, yielding an organic phase (A1, B1) and aqueous phase (A2, B2) for each sample. All four round-bottom flasks were placed in a rotary evaporator for approximately 20 minutes (T=60°C, 130 RPM, 600 mbar, flow=97.7 [I/h]), in order to ensure complete evaporation of organic solvent prior to analysis. After evaporation, extracted aqueous phase samples were made up to 25 mL and extracted organic phase samples were made up to 10 mL, using pure water.

Copper analysis using MPAES (Microwave plasma atomic emission spectrometer): Microwave plasma atomic emission spectrophotometer (Model no. 4100 MPAES) was used to identify concentration of copper in each separated phase. Before calibration, standard solutions were made using copper solution (1000 ppm in ca. M nitric acid copper (II) ion single element solution).

Selection of materials and methods

When formulating solid lipid microparticles, choice of lipids, surfactants, and preparation technique, all affect the physical and chemical characteristics of SLNs. Firstly, selection of lipids is vital as they form the core of the particle itself. The lipids vary from fatty acid chain lengths affecting polarity, melting point and, in turn, stability of the crystallized form. For this experiment, SLNs were made using tristearin as the main lipid, constituting to a phase volume of 0.2 (20% w/v).

Effect of lipid on particle size diameter

Tristearin falls under the class of fats labelled as triglycerides which are class 1 polar lipids [12]. They share the same space with free fatty acids and diglycerides as they all affect surface solubility and have the ability to form stable monolayers [12,13]. Triglycerides are solid-state fats and consist of 3 different polymorphic forms upon crystallization (Table 2). The rate of crystallization is primarily due to the length of the fatty acid chains in their structure. With longer chains, structures upon cooling exhibit different morphologies and result in changes in SLN stability, shape, size, drug entrapment efficiency and drug release profiles [13-15]. These effects are observed upon cooling of the lipid melt, at the beginning of SLN formation.

Lipids are also very sensitive to the methods chosen for formulation, as this can affect particle size and particle behavioural properties. For example, delaying crystallization of lipid melt derived from hot homogenization leads to an increase in particle size, which causes a reduction in suspension stability of the formulation as a whole [16,17]. Therefore, smaller particle sizes improve stability and counteract steric forces to better withhold the suspension. Also, SLNs consisting of less ordered crystals display reduced drug expulsion and drug entrapment as opposed with a homogenous distribution of structure [13,16,18,19]. The crystal structure is dependent on the lipid as it controls how it behaves with temperature changes, also by speed of cooling (Figure 6). In addition, SLN particle aggregation occurs predominantly due to polymorphic transitions of the crystalline SLN whilst in production. Ideally, lipids cannot be used alone in formulating SLNs as other surfactants are needed to compactly pack the SLN structure to prevent systems from aggregating and thus improving stability [1,20].

A small sample, consisting of 3% Tween®20 with 5:1 poloaxmer L68, was made to test the effect of homogenization speed on particle

Copper standards prepared via serial dilutions	Concentration of copper (ppm)	Intensity
Blank (using pure water)	0	0
Standard 1	0.0001	567.53
Standard 2	0.001	961.91
Standard 3	0.01	5007.97
Standard 4	0.1	25658.96
Standard 5	1.0	175611.32

Table 2: MPAES calibrated intensity of copper from standard solutions prepared via serial dilutions.

size in order to establish a concrete homogenization speed for the entire formulating process of SLNs. Two separate batches of the sample were placed under hot high-shear homogenization for 10 mins at speed 3000 RPM and 8000 RPM, respectively. The homogenization speed of the samples was selected at two extremes, low and high, in order to identify a clear trend. Samples exhibited a significant decline in particle size from 235 nm to 154.8 nm with increasing homogenization speed. Hence, homogenization speed of 8000 RPM was chosen and added to the standard SLN preparation protocol for this study 16 samples were extracted at five time intervals of 5, 10, 20, 30, 40 minutes during the homogenization process.

Results and Discussion

Effect of homogenization time on particle size diameter

All samples, on average, exhibited a similar particle size decline from 5-10 minutes of homogenization, which was then followed by an increase in particle size (Table 3). Samples which were extracted after the 10 minute time interval appeared milky and were significantly viscous than samples extracted at 5 minute and 10 minute interval. Comparing overall size distribution characteristics apart from effects of mixture content, increasing homogenization time aids in particle size reduction and narrows width of size distribution [1]. Particles initially under homogenization reach their maximum size reduction at a preliminary time frame, based on the homogenization speed, as with higher speeds the time interval shortens. With added or continued homogenization after that stage, particle size tends to remain constant due to the reduction of size distribution and, as an effect of kinetic movement, it can be assumed that with increasing homogenization time, particle size increases due to particles aggregation [1,21].

Effect of surfactants on particle size

Surfactants' play a key role as additives in the colloidal system. They aid in reducing surface tension and surface energy of the system, thus providing stability to the formulation [20,22]. Various types of surfactants exist consisting of different physical and chemical properties in which determining optimal concentration and type of surfactant impacts the SLN quality. SLN pre-formulations containing mixtures of more than one surfactant has been proven to be more efficacious in terms of stability, viability and generating smaller particle sizes [20]. Hence, a second surfactant was chosen for this system to formulate an optimal SLN for the study. This study focuses on the impact of surfactants on particle size of SLN, involving the use of four non-ionic surfactants Lutrol® F68, F127NF and Tween®20, 80 in different concentrations in the SLN preformulation mixture with tristearin.

Tween®20 (polyoxyethylene sorbitan monolaurate) and Tween®80 (polyoxyethylene sorbitan monooleate) are polysorbates, commonly found in various products ranging from food to pharmaceutical products [20,23]. These molecules differ in their fatty acid chains consisting of the same sorbitan ring backbone with ethylene oxide polymers. The lengths of the fatty acid chains depict the hydrophobic nature of the compound, whilst the hydrophilic nature is derived from the ethylene oxide polymers. With increased concentration of surfactant in formulation enables increased surfactant-particle contact promoting surfactant coverage of interface stabilizing hydrophobic surfaces of SLNs during the polymorphic transition of the incorporated lipid [14,24]. This is achieved by the hydrocarbon chains in the structure as they coat themselves onto the particle allowing for particles to remain in solution longer and promote smaller particle size [14]. However, to determine an optimal concentration for surfactants is difficult, hence this study aimed to cover a range of Tween concentrations of 2, 3, 5 and 10% w/v of both Tween series to observe its effects on particle size.

Forms	α	β'	β_i
Melting point (°C)	54.9 +/- 4	65.3 +/- 0.5	72.4 +/-0.5
Stability profile	metastable	unstable	Stable
Unit structure	hexagonal	orthorhombic	Tricyclic
Particle shape	spherical	needle-shaped	platelet-shaped

Table 3: Characteristics of crystal polymorphic forms of tristearin [13,14,20].

Lutrol® F68 and F127 NF are polyoxyethylene–polyoxypropylene–polyoxyethylene type block copolymers, also known as poloxamers, commonly used as surfactants in various pharmaceutical formulations [25,26]. The different proportions of polyoxyethylene and polyoxypropylene give rise to several different poloxamers differing in molecular weight and hydrophilicity of the surfactant. They improve circulating properties and allow spontaneous self-assembly of vehicle to occur. These were added in a standard 5:1 ratio in accordance with Tween series in the formulation mixture. Since these parameters were kept stable, an ideal indication of effect from the poloaxmer was not well observed, however these ratios did allow comparing concentrating effects of two different poloxamers with a certain Tween concentration.

Samples containing higher concentration of Tween®20 and Tween®80 produced highly viscous solutions and became difficult to analyze with increasing homogenization time, especially in SLN (microparticle) formulations containing 10% Tween®20/80. Tween®80 contains longer fatty acid chains than Tween®20, hence produced immeasurable results at 10% w/v. This can be clearly seen in Figures 2 and 3, where with increasing Tween®80 concentration many samples were unable to be analysed by the zetasizer due to instant solidification of the highly viscous solution. Focusing on the size distribution after 30 minutes, SLN samples containing Tween®20 were able to produce viable data showing an increase in particle size with L127 as opposed to L68. Tween®80 SLN samples containing L68 did not generate accountable samples when 10% w/v Tween®80 was used, corresponding this to the longer chains in the molecule grasping onto the SLN later on aiding to a highly viscous solution. However, with decreased concentrations of Tween®20 and Tween®80 viable data was produced.

A common trend observed in both Tween®20 and Tween®80 particles, is the significant reduction in initial particle size with decreasing Tween concentration, disregarding Lutrol concentration (Figures 4 and 5). SLNs formulated using Tween®80 produced larger particles (>350 nm) after 30 minutes homogenization and also with increasing concentration, in which samples were also immeasurable as compared to Tween®20. The reason behind this could be due to the longer fatty acid chains in Tween®80 stabilizing the hydrophobic regions, in which situations of higher Tween concentrations these can lead to an excess causing accumulation of these surfactants. In addition, with increasing kinetic energy of the solution with homogenization particles have increase motility causing particle-particle interactions more likely. In which case, the longer fatty chains protruding out of the SLN surfaces may cause many to agglomerate together. It can be presumed that the higher particle sizes obtained from the Zetasizer apparatus could be sizes of a cluster of smaller particles forming a large particle as opposed to a single particle.

For Tween® series, as mentioned above, with increasing concentration resulted in highly viscous and milky solutions with increasing homogenization time for both Tween® 20 and 80, generating an increase in particle size. With the incorporation of poloaxmer F127 higher particle sizes were produced in comparison with F68,

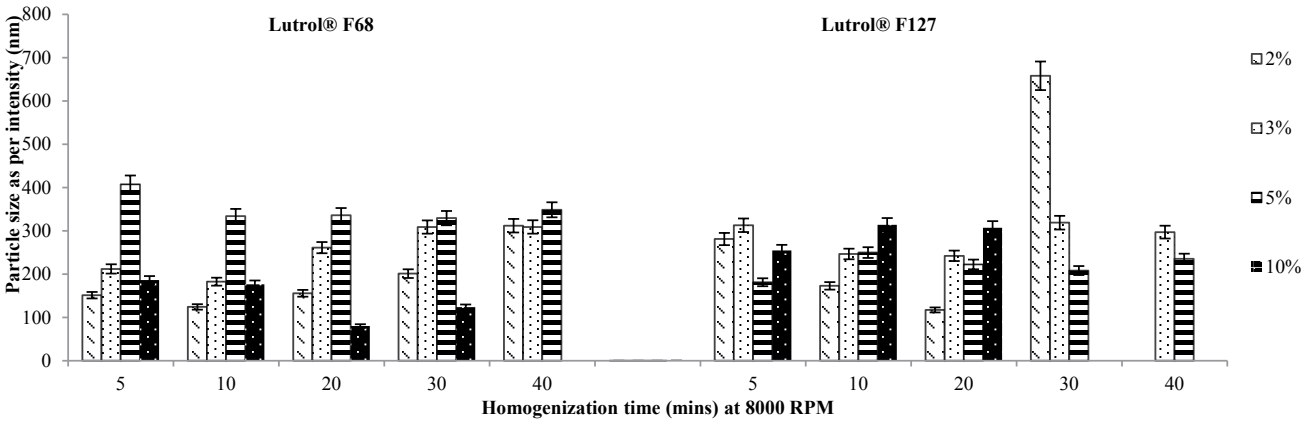

Figure 2: Comparing SLN samples 1-8 consisting of varying concentration s of Tween®20 (2%, 3%, 5%, 10%), with 5:1 ratio of poloxamers Lutrol® F68 and Lutrol®F127. Samples were measured in accordance with 5% standard error.

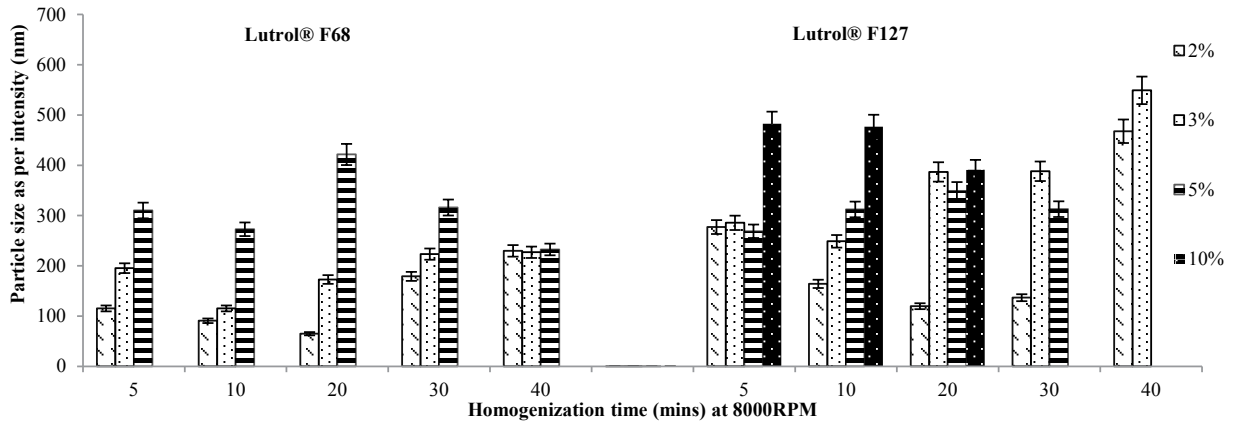

Figure 3: Comparing SLN samples 9-16 consisting of varying concentration of Tween®80 (2%, 3%, 5%, 10%), with 5:1 ratio of poloxamers Lutrol® F68 and Lutrol®F127. Samples were measured in accordance with 5% standard error.

in over 60% of all samples. Samples of F127 collected at 5 min post-homogenization, regardless of percent composition, produced larger particle sizes than with F68 samples (Tables 4 and 5). Results can be explained with the physical properties of poloxamers themselves, allowing steric stability and avoiding aggregation in formulation [26]. Poloxamers contain non-linear hydrophobic chains which comfortably insert themselves into the monolayer of the SLN microparticle. This insertion 'joins' the particle, compacting the SLN by filling void spaces, overcoming steric hindrance, resulting in a reduced particle size. Poloxamers with higher chains, (F127 > F68), have longer PEO terminal chains protruding from the particle after insertion which can potentially explain the larger particle size in F127 compared to F68 [15,27]. Also, upon adsorption of poloaxmer on particle surface, the hydrophilic field on the particle is greatly enlarged-the extent depending on the molecular weight of the poloaxmer- resulting in an increased particle size. In contrast, Lutrol®F127, at high temperatures (greater than body temperature), has the ability of reversible thermal gelation, which becomes reversible upon cooling, producing low level viscous solutions [25]. This can be seen in samples containing 10% w/v of high molecular weight Tween®80 and 5:1 ratio of F68 (~1.75% w/v), particle size measurements were not possible due to the samples being highly viscous from which they solidified upon cooling. However,

Tween® series	Concentration (w/v %)	Lutrol®F68	Lutrol®F127
Tween®20	10%	186.3 nm	254.8 nm*
	5%	407.53 nm	181.2 nm
	3%	211.95 nm	312.95 nm*
	2%	151.29 nm	281.03 nm*
Tween®80	10%	**	482.5 nm
	5%	310.06 nm	268.7 nm
	3%	195.4 nm	285.6 nm*
	2%	115.2 nm	277.6 nm*

[*] indicating significant difference between the two values.
[**] indicating samples were too viscous, and solidified before particle size analysis.

Table 4: Comparing average particle sizes (nm) 5 minutes post-homogenization at 8000 RPM.

Phase partition	Concentration of propionic acid (Ka=1.3 × 10^{-5})	
	0.1M	**1.0M**
Aqueous phase	0.0139 ppm	0.0823 ppm
Organic phase	7.84 × 10^{-3} ppm	3.91 × 10^{-3} ppm

Table 5: Concentration of copper obtained from samples calculated using calibrated equation, y=176446 *x*.

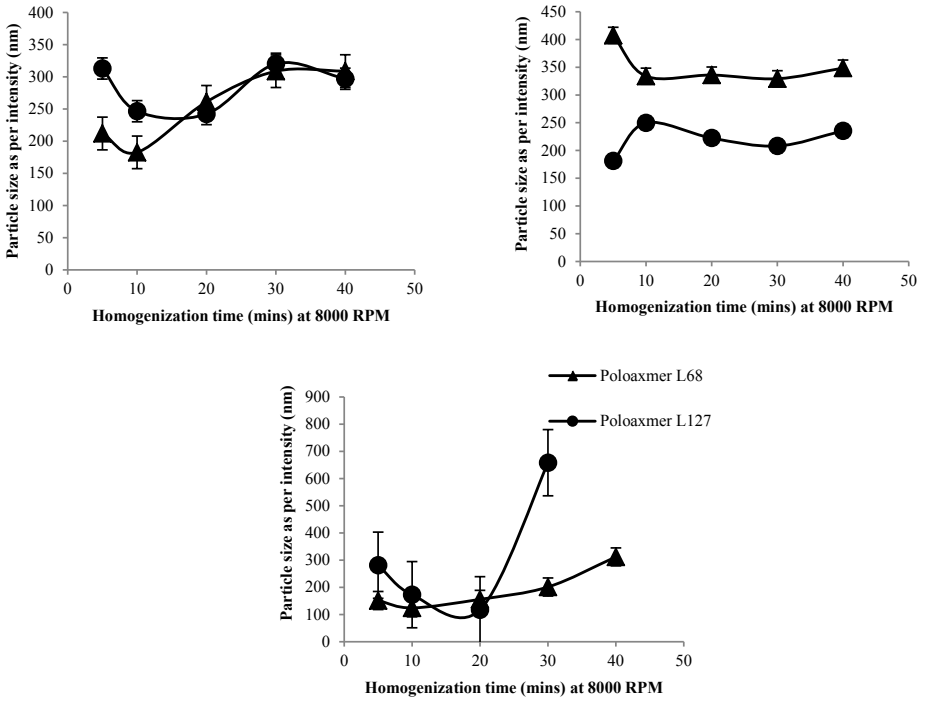

Figure 4: Comparing SLN size of samples containing Tween®20, in descending order of concentration 5%, 3%, 2%, (A, B, C) respectively, observing effects of concentration along with poloxamer type. SLNs formulated using lower Tween®20 concentrations, initial particle size obtained is much smaller. SLN samples (A, B and C) display similar characteristics, as homogenization time increases, particle size increases. These results indicate that reducing concentration of Tween®20 decreases particle size.

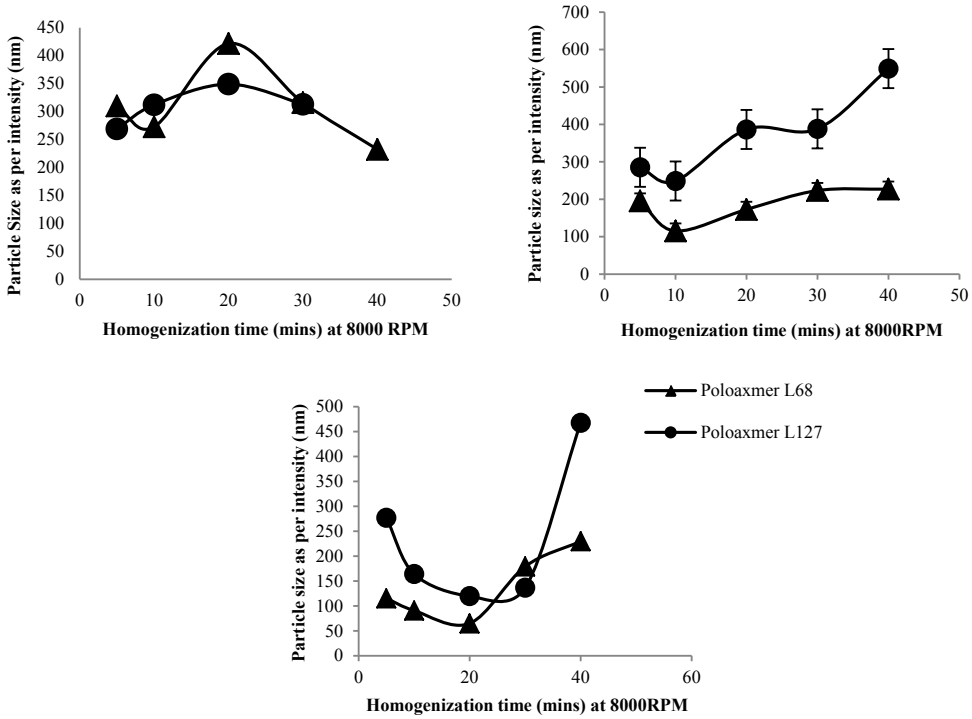

Figure 5: Comparing SLN size of samples containing Tween®80, in descending order of concentration 5%, 3%, 2%, (D, E, F) respectively, observing effects of concentration along with poloxamer type. Similar to that of Tween®20, with decreasing Tween concentration, initial particle size decreases. Poloxamer L127 shows significant increase in size with increasing homogenization time.

the same sample containing F127 was able to be measured due to the system being less viscous. The brush-like behaviour of the poloaxmer itself can aid in the bulkiness of the solution but when added alongside Tween series, potentially Tween®80, the effect is increased due to the similar nature of the protruding fatty acid chains [28,29].

The results obtained cannot solely be based on surfactant properties, where the lipid constituent may also affect the size distribution characteristics observed here. As mentioned previously, transition phases of the lipid melt have significant effects on the microparticle as a whole altering both morphological and release kinetic properties of the SLN. The lipids are extremely sensitive to preparation methods endorsed where varying temperatures can effect crystallization of the SLN. In addition, when samples were analysed via SEM, crystallization effects on the particle were visible with increasing homogenization time (Figure 6). For example, when comparing SLN samples constituting of Tween®80 and F68 at homogenization time 10 minutes and 20 minutes, SLNs viewed at 20 minutes displayed platelet/needle-like shape characteristics. This can either be seen as the lipid entering β' or β_i form, away from the metastable α (spherical shape). Considering temperatures kept above 70°C it can be assumed particle exhibiting the stable β_i, however this can only be confirmed with further analysis of the particle [30-32].

Sample 15 was determined as the optimal concentration for this study. The mixture containing,

- 80% w/v Tristearin
- 20% phosphate buffer
- 2% w/v Tween®80
- 5:1 Lutrol F68

Produced the aimed particle size between 90-115 nm at 5-10 minutes of homogenization. In comparison with 2% Tween®20, average microparticle size ranged from 120 nm-150 nm. The reason behind this phenomenon could be due to the longer fatty acid chains in Tween®80, providing better coverage of the hydrophobic regions in/on the SLN with low concentration as opposed to Tween®20 thus, resulting in smaller particle size.

Analysis of hydrophobised copper particles

Propionic acid was used as a method to potentially coat the copper nanoparticles in hopes of allowing the moiety to become more hydrophobic. As mentioned previously, Copper (II) nitrate solution was used as a standard to perform a calibration plot on the copper samples (Figure 7). The aim was to develop a successful coating mechanism to lyophilise positively charged copper nanoparticles in order to sustain in the highly lipid SLN structure. In order to quantify the magnitude of partition, the hydrophosied copper was separated between an organic solvent (diethyl ether) and an aqueous solution (pure water). Firstly, two separate concentrations of propionic acid were made, 0.1 M and 1.0 M, obtaining a final concentration of copper in both solutions 0.398 ppm after serial dilutions. Upon addition of the organic solvent, the flask was stirred to allow contents to interact, after which the two phases were extracted into separate round bottom flasks. A MPAES analysis was conducted to quantify unknown copper concentration using standards formulated in the lab for the calibration. The results showed a suitable amount of copper present in the organic phase (as hoped), however higher traces of copper were found in the aqueous phase of both acid concentrations.

Future Prospects

This project aimed to identify changes in SLN particle size from surfactants Tween®20, Tween®80, Lutrol® F68 and Lutrol® F127, in the direction of finding the optimal formulation from these surfactants to produce SLN sizes under 200 nm. Further work can be conducted in using different lipids and comparing with tristearin, along with changing phase volume ratios to evaluate effect on particle size, stability, shape. From present work, a 2% w/v Tween®80 concentration produced desired particle sizes of 120-150 nm. Additional work can include testing detailed parameters of these concentrations, using reduced Tween concentration to better achieve a lower particle size. In addition, different surfactants can also be tested for example, Span and other poloxamers etc.

The use of copper was a secondary feature in this experiment. The idea of formulating a coating system around copper was a means of allowing metal incorporation into the SLN for formation of a novel brachytherapy "seed". The nanometre size range of the SLN system would provide great benefit as opposed to the conventional brachytherapy seed (Figure 8). The use of copper was representative to that of radioisotopes used in the treatment, thus enabling safe *in vitro* studies. The main concept was to establish a successful method to obtain enhanced lipophilic ability. The results did comply with the objective, however was not significantly sufficient to label the method as successful. Further work can assess different acids, bases, or even different formulating strategies for copper coating. The next step would be incorporation into the SLN, examining size, shape, release kinetics of the particle.

Conclusions

In the present work, SLNs derived from higher Tween®20/80 concentrations (>5%) were difficult to analyse as mixture completely solidified upon cooling, hence failed. A possibility of large particle aggregation and stronger molecular attraction can be assumed as the result. Also, majority of samples depicted a decrease in particle size with increased homogenization time up to a limit. Homogenization post 10 min significantly increased particle size. Incorporation of larger poloaxmer (F127) also aided in the microparticle size increased, where it was found using low w/v% of surfactants and smaller poloxamers resulted in a reduced particle size.

The underlying principle of any drug formulation is that the designed vehicle must ordain patient safety. SLNs satisfy this by their biocompatible and biodegradable formulating ingredients, initiating the first step towards development. As outlined previously, they do tackle the best and eliminate the worst of the many conventional colloidal drug carrier systems. The ability of solid lipid microparticles to function as its proposed mechanism is solely based their ingredients. Pre-formulation techniques and methods need to be considerably researched and tested prior to production, providing an optimal SLN. Research is still ongoing in terms of finding an optimal mixture of surfactants to lipids, also identifying key surfactants which generate desired particle size. With further research these grey areas will become well explained.

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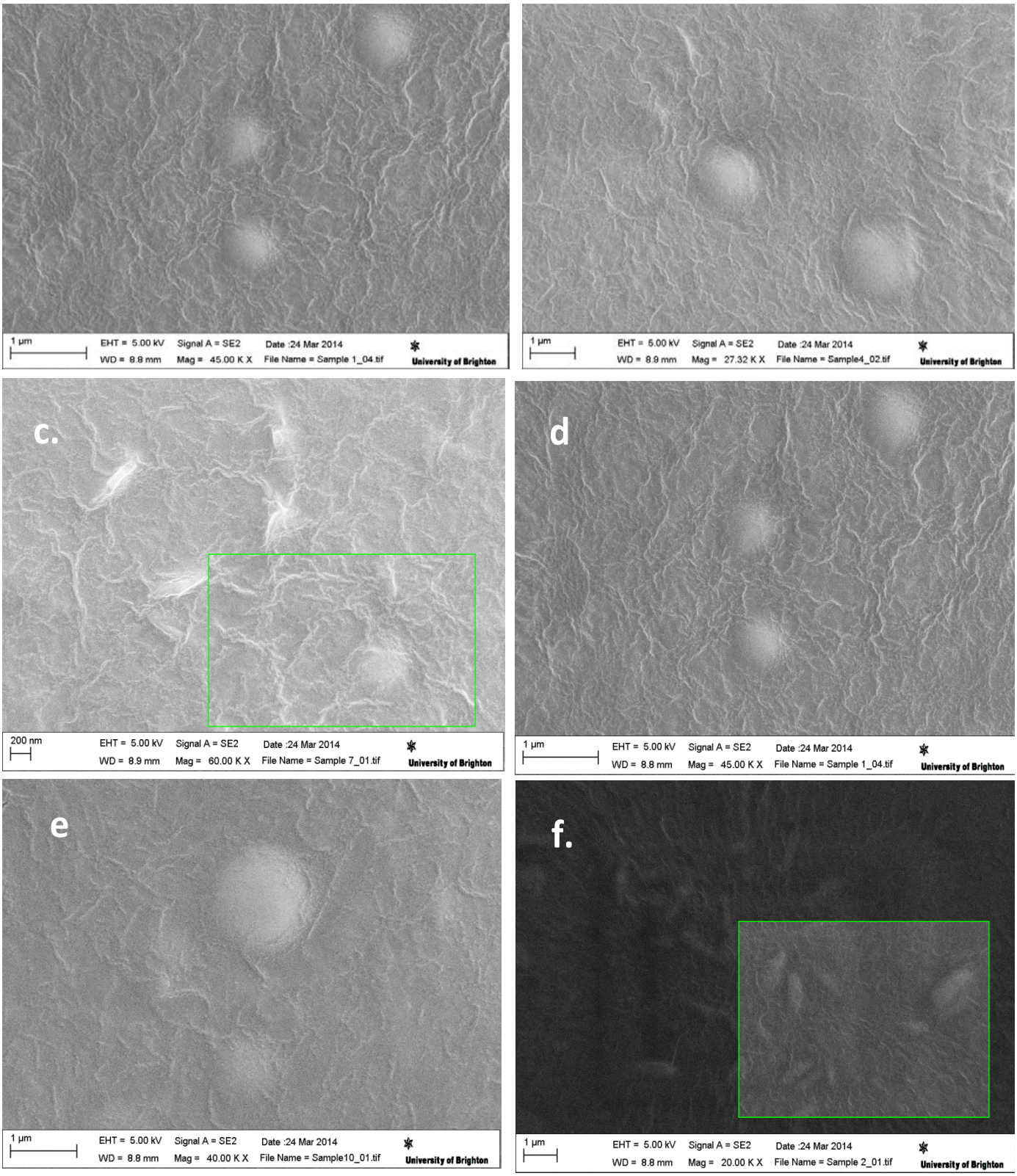

Figure 6: SEM analysis of samples, with varying magnification, corresponding to 10 min homogenization time at 8000 RPM with Tween® 20 and 80 concentration at 2% w/v, comparing (a) T80+F68 with, (b) T80+F127, of an average particle size of 90.82 nm and 164.13, respectively; (c) T20+F68 with, (d) T20+F127, of an average particle size 124.3 nm and 172.93, respectively; (e) T80+F68 post 10 mins homogenization, (f)T80+F68 post 20 mins homogenization, indication of polymorphic phase transition of lipid upon crystallization to β' and/or β_1 form.

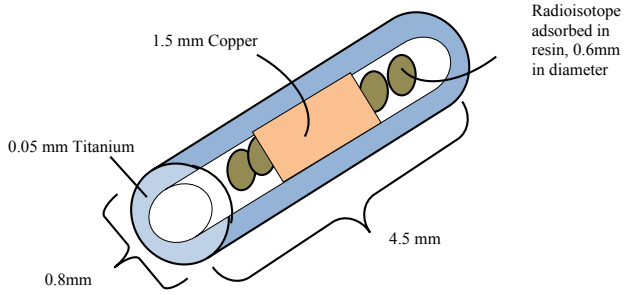

Figure 7: Calibration curve of copper standards prepared via serial dilution of copper (II) nitrate solution (Table 2), generating R^2=0.9969, at Cu 324.7 λ, using MPAES.

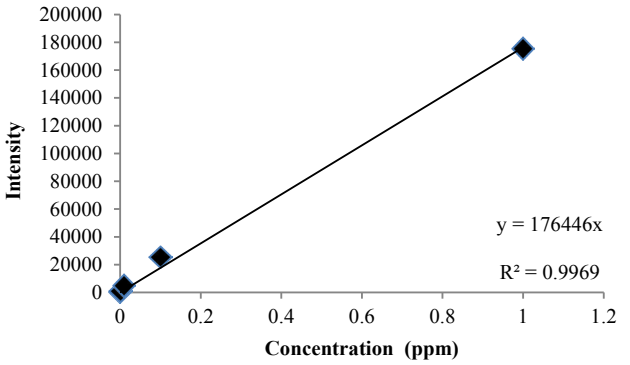

Figure 8: Representing the current model of the brachytherapy seed [31].

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