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# Optimisation and Stability Assessment of Solid Lipid Nanoparticles using Particle Size and Zeta Potential

Rohan Shah,* Daniel Eldridge, Enzo Palombo and Ian Harding

Department of Chemistry and Biotechnology,
Faculty of Science, Engineering and Technology, Swinburne University of Technology,
P.O. Box 218, Hawthorn VIC 3122, Melbourne, Australia

*Corresponding author: rshah@swin.edu.au

**Abstract:** *Solid lipid nanoparticles (SLNs) are a well-tolerated lipid carrier system due to the employment of a physiological and/or biodegradable lipid matrix. Physicochemical properties such as particle size, polydispersity index (PI) and zeta potential are SLN quality response parameters. Increased particle size is a good indicator of in-vitro instability. This work focuses on the importance of selecting the lipid matrices and excipients that can achieve the particle size and stability required if such formulations are to be utilised in the pharmaceutical market. With the aim of understanding the influence of variation in SLN composition (lipid and emulsifier concentration), a Taguchi model of experimental design was applied. Tested factors included the concentration of lipid (stearic acid) and the concentration of Tween®20. SLNs were successfully prepared by a microemulsion-based technique. Based on the hydrophilic lipophilic balance (HLB), different combinations of emulsifiers/co-emulsifiers (Tween®20/Span®20, Tween®20/Span®80, Tween®20/n-butanol, and Tween®20/iso-propanol) were also used to control the physicochemical properties of SLNs. The influence of pH (addition of HCl or NaOH) and electrolyte (addition of NaCl), both during and after the preparation, were also investigated on selected SLN formulations. Slightly polydispersed (PI < 0.3) nanoparticles with a particle size < 450 nm and zeta potential range of +5 to –50 mV were developed. Physical stability of optimised stearic-acid based SLNs over 2 months were assessed by particle size measurement. SLNs were stable when refrigerated. These results suggest that thoughtful selection of lipid and lipid excipients is essential for successful preparation and physical stability of SLNs. This study facilitates the preliminary physicochemical characterisation for favourable encapsulation of lipophilic and hydrophilic drugs.*

**Keywords:** Solid lipid nanoparticles, zeta potential, hydrophilic lipophilic balance, drug encapsulation, lipophilic and hydrophilic drugs

## 1. INTRODUCTION

Approximately 40% of commercialised drugs are poorly water-soluble, making it difficult to obtain adequate and reproducible drug absorption from the gastrointestinal tract.[1] Low solubility and variable drug absorption lower the drug bioavailability and eventually lead to compromise in the drug efficacy and

safety.[2] A few colloidal carriers (micellar solutions, liposomes, nanoemulsions, nanosuspensions and polymeric nanoparticles) have been previously investigated as probable carriers to overcome the issues related to solubility and bioavailability of drugs. However, certain drawbacks such as limited physical stability, low drug loading, drug expulsion on storage, presence of residual organic solvents and polymer cytotoxicity are associated with these colloidal carriers.[3]

This led to the emergence of novel solid lipid nanoparticle (SLN)-based carriers. SLN-based colloidal carriers have gained an increasing attention in drug delivery. Use of physiological lipids that are solids at room and body temperature promotes drug absorption primarily via improved drug dissolution and solubilisation in the intestinal milieu, enhanced lymphatic transport, reduced gastric emptying rate and increased gastrointestinal permeability. SLNs potentially incorporate the benefits of other carrier systems whilst reducing their documented shortcomings.[4]

SLNs are colloidal systems with particles typically in the size range of 50–1000 nm and composed of biocompatible and biodegradable solid lipids, well-tolerated in physiological systems and generally recognised as safe (GRAS).[5] This nature of SLNs confers specific distinct advantages such as:[6–8]

1. Ability to encapsulate and protect labile drugs from degradation
2. Improved bioavailability of poorly water-soluble drugs
3. Ability to modulate the drug release and drug targeting
4. Excellent physical stability
5. Feasibility of large scale production and sterilisation

For these various reasons, SLNs have emerged as an attractive alternative to other colloidal carriers that can be administered through different routes such as parenteral, oral, dermal and ocular.[6,9–12]

Particle size and size distribution (usually indicated by polydispersity index, PI) are important characteristics that are crucial in the production and stability of SLNs.[13] These characteristics largely depend upon the preparation method and the particle composition.

In the present work, SLNs were prepared by a microemulsion-based technique. Stearic acid was the lipid material selected for the preparation of SLNs. The aim of this work is to systematically investigate the influence of amount of lipid with and without other lipid excipients on the final SLN particle size, PI and zeta potential, and physical stability on short-term storage. The

stability behaviour in terms of particle size measurements of the SLN formulations with respect to time at various temperatures was studied.

## 2. EXPERIMENTAL

### 2.1 Materials

Stearic acid was purchased from Sigma-Aldrich (Australia). Similarly, Tween®20 and Lutrol®F68 were purchased from Sigma-Aldrich. Span®20 and Span®80 were purchased from Merck. All other chemicals were of analytical grade or equivalent. Ultra-purified water was obtained by reverse osmosis from a MilliQ® Plus, Millipore® system (Schwalbach, Germany).

### 2.2 Preparation of SLNs

A Taguchi model of experimental design was used to prepare SLNs with varying amounts of stearic acid and Tween®20 before selection of the optimised formulation for further study.[14,15] The amount of stearic acid ($X_1$) and the concentration of Tween®20 ($X_2$) were the different parameters investigated (Table 1). The total volume of the dispersion was set at a fixed level. Table 2 gives the composition of different formulations prepared using the Taguchi model. The experiments were performed in a random fashion to avoid any systematic bias in the results.

Table 1: Levels of the factors in the Taguchi model of experimental design for preparation of SLNs.

| Parameters | Levels | | |
|---|---|---|---|
| | –1 | 0 | –1 |
| $X_1$ – Amount of stearic acid (mg) | 50 | 75 | 100 |
| $X_2$ – Concentration of Tween®20 (%) | 0.15 | 0.20 | 0.25 |

The SLNs were prepared by a novel microemulsion-based technique. Briefly, the molten lipid material and heated aqueous surfactant solution were mixed at a temperature above the melting point of stearic acid (approximately 85°C). Energy was provided to the system in order to form the emulsion and the microemulsion obtained was immediately dispersed in cold water (initially held at 2°C–5°C), with continuous magnetic stirring, to obtain SLN dispersion. The formulations were further subjected to particle size analysis and zeta potential measurements.

Table 2: Taguchi design for preparation of stearic acid-based SLNs.

| Formulation code | Factors (Composition) | | Responses | | |
|---|---|---|---|---|---|
| | Amount of stearic acid ($X_1$, mg) | Concentration of Tween® 20 ($X_2$, %) | PS (nm) ± SD | PI ± SD | ZP (mV) ± SD |
| F1 | 50 | 0.15 | 266.9 ± 13.7 | 0.228 ± 0.008 | –16.20 ± 0.02 |
| F2 | 50 | 0.20 | 151.8 ± 15.8 | 0.214 ± 0.062 | –11.01 ± 1.24 |
| F3 | 50 | 0.25 | 147.0 ± 3.5 | 0.208 ± 0.016 | –14.38 ± 0.82 |
| F4 | 75 | 0.15 | 330.7 ± 29.1 | 0.273 ± 0.033 | –15.12 ± 1.00 |
| F5 | 75 | 0.20 | 206.1 ± 5.3 | 0.198 ± 0.004 | –14.52 ± 0.42 |
| F6 | 75 | 0.25 | 175.6 ± 0.6 | 0.177 ± 0.008 | –14.55 ± 0.78 |
| F7 | 100 | 0.15 | 372.2 ± 19.9 | 0.213 ± 0.018 | –15.95 ± 0.38 |
| F8 | 100 | 0.20 | 259.8 ± 20.6 | 0.202 ± 0.037 | –14.17 ± 0.19 |
| F9 | 100 | 0.25 | 212.1 ± 3.5 | 0.215 ± 0.016 | –14.95 ± 0.18 |

*PS – particle size, PI – polydispersity index, ZP – zeta potential, SD – standard deviation.*

## 2.3 Particle Characterisation

### 2.3.1 Particle size analysis

Particle size analysis of the SLNs was performed by dynamic light scattering (DLS), also known as photon correlation spectroscopy (PCS), using a 90Plus Particle Size Analyser (Brookhaven Instruments Corporation, New York, USA). Prior to the measurements, all formulations were diluted using ultra-purified water to yield an appropriate scattering intensity. Particle size measurements were carried out at 25°C. The particle size and PI of the investigated formulations were obtained by calculating the average of 10 measurements at an angle of 90°.

### 2.3.2 Zeta potential measurements

The zeta potential of the SLNs was determined by the measurement of the electrophoretic mobility using a 90Plus Particle Size Analyser (Brookhaven Instruments Corporation, New York, USA). The conversion of the electrophoretic mobility to zeta potential was performed using the following Helmoltz-Smoluchowski equation:[2]

$$\zeta = E\,(4\pi\eta/\varepsilon)$$

where,

$\zeta$ = zeta potential (mV)
E = electrophoretic mobility
$\eta$ = viscosity of the dispersion medium (water 0.8904 cp)
$\varepsilon$ = dielectric constant of the solvent (water, 78.54)

Whilst it is well known that the Helmoltz-Smoluchowski equation results in errors for particles in the size range of the microemulsions formed here, the values are used as qualitative guides to particle stability rather than quantitative assessment of the zeta potential. A more vigorous analysis of zeta potential is yet to be performed.

Prior to the electrophoretic mobility measurements, all the samples were diluted with ultra-purified water, and the measurements were carried out at 25°C.

## 2.4 Formulation Optimisation

The influence of different formulation factors on the particle size and zeta potential was investigated. The optimal results of each formulation factor for stearic acid-based solid lipid nanoparticles were used in subsequent experiments. Unless otherwise stated, the standard preparation method described earlier was employed.

### 2.4.1 Influence of pH

Stearic acid (75 mg) was the lipid material used and Tween®20 (0.25%) was the emulsifier used in the following experiments unless otherwise mentioned. To evaluate the influence of pH, formulations with the addition of either 0.1 M NaOH (formulation A) or 0.1 M HCl (formulation B) at two different stages of preparation were evaluated. Influence of the addition of HCl (or NaOH) before preparation implies the inclusion of HCl (or NaOH) in the hot aqueous solution, while the influence of HCl (or NaOH) addition after preparation implies inclusion of HCl (or NaOH) in the cold water.

The influence of the addition of either 0.1 M NaOH or 0.1 M HCl before (formulations A1 and B1, respectively) and after (formulations A2 and B2, respectively) the preparation was studied. In these experiments, the final concentration of either of these solutions was 0.20%. The formulation F6 served as the control to which neither 0.1 M NaOH nor 0.1 M HCl was added. All formulations were subjected to particle size analysis and zeta potential measurements.

### 2.4.2 Influence of alteration of ionic strength

Stearic acid (75 mg) was the lipid material used and Tween®20 (0.25%) was the emulsifier used in the following experiment unless otherwise mentioned. To evaluate the influence of the alteration of ionic strength, formulations with the addition of 0.1 M NaCl (formulation C) at two different stages were evaluated. Influence of the addition of NaCl before preparation implies the inclusion of NaCl in the hot aqueous solution, while the influence of NaCl addition after preparation implies inclusion of NaCl in the cold water.

The influence of addition of 0.1 M NaCl before (formulation C1) and after (formulation C2) the preparation was studied. In these experiments, the final concentration of this solution was 0.20%. The formulation F6 served as the control to which 0.1 M NaCl was not added. All formulations were subjected to particle size analysis and zeta potential measurements.

### 2.4.3 Influence of co-emulsifiers

Stearic acid (75 mg) was the lipid material used and Tween®20 (0.25%) was the emulsifier used in the following experiment unless otherwise mentioned. To evaluate the influence of the co-emulsifiers, formulations with and without the existence of co-emulsifiers were evaluated. The studied co-emulsifiers include Span®20 (formulation D1), Span®80 (formulation D2), n-butanol (formulation D3) and iso-propanol (formulation D4). In these experiments, the total concentration of emulsifiers (with or without co-emulsifiers) was maintained at 0.25%. The final concentration of 0.1 M NaOH in the formulations was 0.20%. The proportion of emulsifier and co-emulsifier was based on the hydrophilic-lipophilic balance (HLB) system. The formulation A1 served as the control. All formulations were subjected to particle size analysis and zeta potential measurements.

### 2.4.4 Influence of stabiliser

Stearic acid (75 mg) was the lipid material used and Tween®20 (0.25%) was the emulsifier used in the following experiment unless otherwise mentioned. To evaluate the influence of the stabiliser, formulations with and without the existence of stabilisers were evaluated. The stabiliser used in this study was Lutrol®F68 (formulation E). In these experiments, the concentration of Tween®20 was maintained at 0.25% and the concentration of the stabiliser was 1%. The final concentration of 0.1 M NaOH in the formulations was 0.20%. The formulation A1 served as the control. All formulations were subjected to particle size analysis and zeta potential measurements.

### 2.5 Short-term Stability Studies

The initial particle size analysis of the stearic acid-based SLN dispersions was performed using the 90Plus Particle Size Analyser (Brookhaven Instruments Corporation, New York, USA) as described earlier (See section 2.3). This batch was divided into three sample sets, one stored at 4°C (in a refrigerator), the second stored at 25°C and the third stored at 37°C (both in temperature-regulated incubators). All samples were stored in plain sealed glass vials. Samples were removed after 4, 15, 30, 45 and 60 days and subjected to particle size measurements.

### 2.6 Statistical Analysis

The results were expressed as mean ± standard deviation. Statistical analysis was performed on the data sets with analysis of variance (ANOVA). Differences were considered significant for $p < 0.05$.

## 3. RESULTS AND DISCUSSION

Several methods have previously been reported for the preparation of SLNs, such as high sheer homogenisation, high pressure homogenisation, solvent diffusion, solvent evaporation and solvent injection methods.[6] A simple microemulsion-based method, with no toxic organic solvents and amenable for up-scale production, was used in the current study. Preliminary formulation studies included selection of the appropriate amount of stearic acid in combination with Tween®20.

### 3.1 Particle Characterisation

#### 3.1.1 Particle size analysis

Particle sizing is an important characterisation technique to confirm the production of nano-sized particles. DLS presents the particle size of the SLNs as hydrodynamic diameter (intensity weighted mean diameter, or the z-average diameter) and the PI as an indication of the width of the particle size distribution. The PI value that reflects the quality of the dispersion usually ranges from 0 to 1. PI values $\leq 0.1$ indicate the highest quality of dispersion. Most researchers recognise PI values $\leq 0.3$ as optimum values; however, values $\leq 0.5$ are also acceptable.[16]

Table 2 gives an overview of the formulations prepared using the Taguchi design. The particle size and the PI values for all the formulations have

been evaluated by DLS. The particle size of the SLNs prepared was in the range of 125–500 nm, indicating a significant influence of formulation variables on the resultant particle size. Statistical analysis of the data suggested that the proposed design was significant ($p < 0.05$). The prepared SLN dispersions had a PI value $\leq 0.25$ indicating a homogenous distribution of SLNs.

### 3.1.2 Zeta potential

Zeta potential, which can be either positive or negative in polarity depending upon the chemistry of the particles, is an electric potential created by the presence of a charge on the particle surface. Zeta potential is an indicator of the degree of repulsion between similarly charged particles in the formulation. Repulsive forces prevent particle aggregation during storage. Zeta potential is thus indicative of probable physical stability of a formulation.[17]

Table 2 reports an overview of the results of the zeta potential measurements. The zeta potential of the different formulations was consistently negative and in the range –11 to –17 mV. No linear correlation was observed between the zeta potential values and either the amount of stearic acid used or the concentration of Tween®20 in the formulation.

SLNs perfectly covered by a non-ionic surfactant like Tween®20 tend to remain stable despite having a lower zeta potential. Greater steric stabilisation and less electrostatic stabilisation are responsible for such behaviour. Surface coverage of the SLNs reduces the electrophoretic mobility of the particles and thus lowers the zeta potential.[18] Hence, zeta potential measurement was not considered a primary parameter in the selection of the optimal formulation.

## 3.2 Selection of the Lipid Type and Amount and Concentration of Tween®20

As previously discussed, particle size, PI value and zeta potential measurements are important parameters in optimising the formulation. In the preliminary experiments for the selection of formulation constituents and their concentration for further optimisation, particle size and PI were selected as the most important response parameters.

### 3.2.1 Influence of amount of stearic acid

Stearic acid was used as the lipid material in the preparation of SLNs. The results obtained are given in Table 2. The results clearly indicate that the amount of stearic acid has a positive influence on the mean size of the SLN, i.e., larger SLNs at higher amounts of stearic acid (Figure 1). It is logical that an

increased particle size is observed with higher amounts of stearic acid. Lack of sufficient surfactant to cover the particle surface is the likely reason for increased particle size.[19]

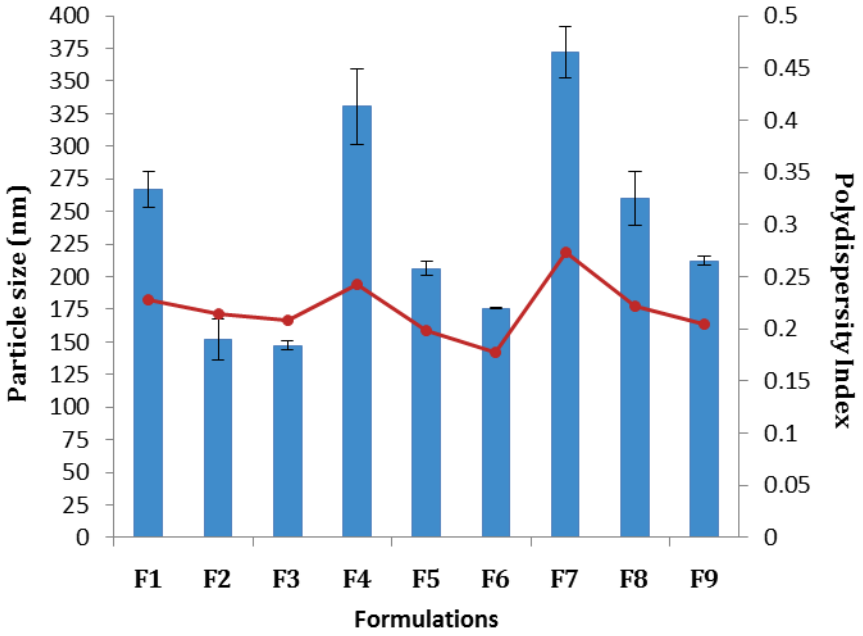


Figure 1: Influence of amount of stearic acid and concentration of Tween®20 on particle size (solid bars) and polydispersity index (red dots). Formulation codes (on X axis) are as per Table 2.

Increased particle size may be due to the influence of increased viscosity.[20] Higher amounts of stearic acid increase the viscosity of the inner phase and affect the shearing capacity of the stirrer (size reduction becomes difficult). As a result, particles tend to increase in size.[21]

### 3.2.2 Influence of concentration of Tween®20

Varying amounts of Tween®20 (0.15%–0.25%) were added to varying amounts of stearic acid. Tween®20 is a non-ionic surfactant with an HLB value of 16.7. Due to its relatively low critical micelle concentration (about 0.06 mM), the concentration of Tween®20 monomers in the dispersion medium is reasonably low. Surfactant monomers, rather than micelles, adsorb onto the hydrophobic surfaces of the fatty acids. This property results in surfactants failing to stabilise the SLNs against particle aggregation and growth even at higher concentrations of Tween®20.[22]

It can be noted that at a constant amount of stearic acid, the concentration of Tween®20 had a negative influence on the mean diameter of the SLNs, i.e., particle size dramatically decreased with increasing concentration of Tween®20 (Figure 1). Higher concentrations of surfactant allow better stabilisation of the smaller lipid droplets and thus prevent them from coalescing into larger droplets.[23]

At higher concentrations, sufficient surfactant present at the surface of the SLN reduces the surface tension between the two phases and enables SLN formation when the hot microemulsion was rapidly injected into the cold water.[18]

### 3.3 Formulation Optimisation

The particle size and the physical stability of the SLN dispersion is influenced by many other formulation parameters such as co-emulsifiers, acids (or bases or electrolytes) and stabilisers.

#### 3.3.1 Influence of pH

The influence of pH before and after the preparation of SLNs was investigated by adding a small volume of either base (0.1 M NaOH) or acid (0.1 M HCl) to the system. The effects of these solutions on particle size analysis and zeta potential measurements and the pH of the formulations are shown in Table 3. The particle size of the formulations increased after addition of each of these solutions. The PI values, however, showed an opposing trend. Addition of acid to the system increased the PI value while addition of base decreased the PI values as compared to the control formulation. In the case of zeta potential, addition of base increased the zeta potential. Addition of acid lowered the zeta potential, which correlates with the higher PI values observed in these formulations.

A minimum zeta potential of about –60 mV yields a formulation with excellent physical stability, while a zeta potential of approximately –30 mV yields a formulation with fairly good physical stability.[23] The zeta potential of A1 was found to be –40.2 ± 1.43 mV. As this formulation exhibited the zeta potential of highest magnitude, A1 was selected for further optimisation.

Table 3: Influence of pH.

| Formulation code | pH ± SD | Responses | | |
|---|---|---|---|---|
| | | PS (nm) ± SD | PI ± SD | ZP (mV) ± SD |
| A1 | 8.49 ± 0.06 | 338.3 ± 8.1 | 0.147 ± 0.017 | –40.20 ± 1.43 |
| A2 | 8.85 ± 0.06 | 385.8 ± 2.5 | 0.113 ± 0.010 | –29.29 + 3.39 |
| B1 | 4.43 ± 0.11 | 419.4 + 7.6 | 0.249 ± 0.033 | –8.97 ± 0.01 |
| B2 | 4.75 ± 0.07 | 414.9 ± 12.2 | 0.312 ± 0.014 | –8.71 ± 0.09 |

*PS - particle size, PI - polydispersity index, ZP - zeta potential, SD - standard deviation, A1 - formulation to which 0.1 M NaOH was added before preparation of SLNs, A2 - formulation to which 0.1 M NaOH was added after preparation of SLNs, B1 - formulation to which 0.1 M HCl was added before preparation of SLNs, and B2 - formulation to which 0.1 M HCl was added after preparation of SLNs*

### 3.3.2 Influence of an electrolyte

The influence of the addition of an electrolyte before and after the preparation of SLNs was investigated by the addition of a small volume of 0.1 M NaCl. According to the results shown in Table 4, the particle size and PI values of the formulations increased upon addition of an electrolyte. The zeta potential of the system after the addition of an electrolyte did not improve to a great extent.

Table 4: Influence of addition of an electrolyte.

| Formulation code | Responses | | |
|---|---|---|---|
| | PS (nm) ± SD | PI ± SD | ZP (mV) ± SD |
| C1 | 402.0 ± 10.7 | 0.320 ± 0.015 | –12.83 ± 1.19 |
| C2 | 324.9 ± 3.7 | 0.280 ± 0.021 | –13.18 ± 0.14 |

*PS - particle size, PI - polydispersity index, ZP - zeta potential, SD - standard deviation, C1 - formulation to which 0.1 M NaCl was added before preparation of SLNs, and C2 - formulation to which 0.1 M NaCl was added after preparation of SLNs*

### 3.3.3 Influence of the co-emulsifiers

The effects of the co-emulsifiers such as Span®20 (HLB = 8.6), Span®80 (HLB = 4.3), n-butanol (HLB = 7.0) and iso-propanol (HLB = 7.4) were also investigated. Matching the HLB of the emulsifier (with or without co-emulsifiers) to the "required HLB" of the lipid material used helps in the preparation of finely dispersed and physically stable formulations. A combination of emulsifiers with their combined HLB value matching the required HLB value usually gives a more stable product.[20]

The required HLB value of stearic acid used in this study is 15. Based on the HLB system, and matching the HLB value to the "required value" of 15, combinations of Tween®20/Span®20 (79:21), Tween®20/Span®80 (86.25:13.75),

Tween®20/n-butanol (82.5:17.5) and Tween®20/iso-propanol (81.75:18.5) were added to the system.

Figure 2 outlines the results of particle size analysis after addition of a co-emulsifier. The particle size was found to increase after the addition of co-emulsifiers, whilst there was a reduction seen in the PI values. This is logical since the combination of co-emulsifiers tends to produce a more stable and dispersed product. The zeta potential of these formulations was in the range of –30 to –40 mV (data not shown).

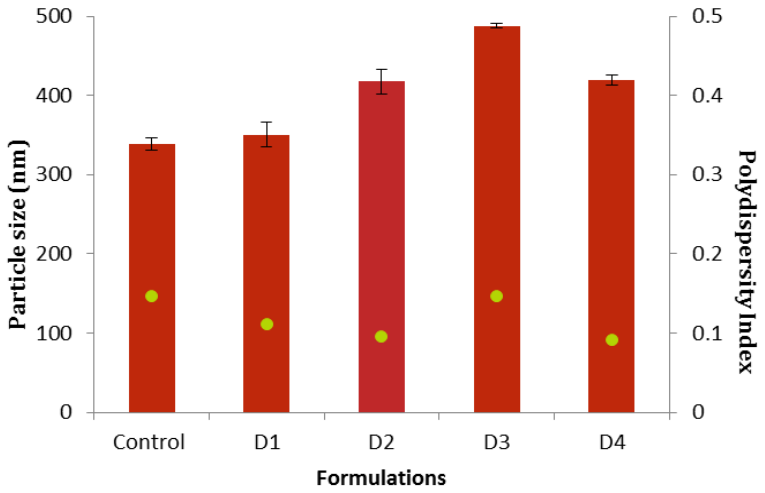


Figure 2: Influence of co-emulsifiers on particle size (solid bars) and polydispersity index (green dots). D1 - Span®20, D2 - Span®80, D3 - n-butanol and D4 - iso-propanol used as co-emulsifiers.

### 3.3.4 Influence of stabiliser

The effect of stabiliser was evaluated by adding two different stabilisers to the dispersion medium. Matching the HLB of the stabiliser with that of the internal lipid phase gives a product with greater stability. The HLB value of Lutrol®F68 is ~28. Figure 3 gives the results of the particle size analysis and zeta potential. An increase in particle size and PI value was observed in formulation E. This could be due to the large difference between the HLB values. Besides, higher molecular weight of Lutrol®F68 (~12600) could be another reason for increased particle size seen in formulation E. Presence of larger molecules of Lutrol®F68 on the surface of SLN contributes to larger particle size of the SLN. This is consistent with the explanation provided by Martins et al.[18] The lower zeta potential also suggests that the use of Lutrol®F68 as a stabiliser yields an

unstable formulation as reduction in the zeta potential is indicative of a less stable formulation compared to the control formulation.

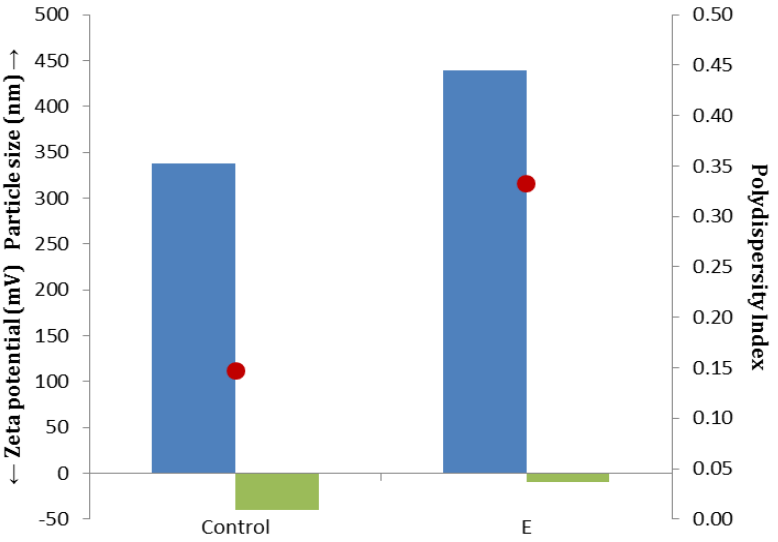


Figure 3: Influence of stabiliser on particle size (blue solid bars, positive values), polydispersity index (red dots) and zeta potential (green solid bars, negative values). E–Lutrol®F68 was used as stabiliser.

### 3.4 Short-term Stability Studies

In terms of selecting the optimal formulation, A1 was selected for short-term stability studies. The physical stability of the optimised formulation was evaluated at 4°C, 25°C and 37°C for 60 days by particle size measurements (Figure 4).

The SLNs stored at refrigerated conditions were stable over a period of 2 months. There was negligible increase in particle size. However, when stored at 25°C, after an initial increase in particle size, further particle growth was not observed after 4 days of storage. The particle size increased to about 714 nm after 4 days and 780 nm after 45 days. The formulation was unstable when stored at 37°C. Storage at 37°C induced rapid particle growth within 4 days of storage. The mean particle size (338.3 nm on day 0) increased to about 1574.8 nm in 4 days.

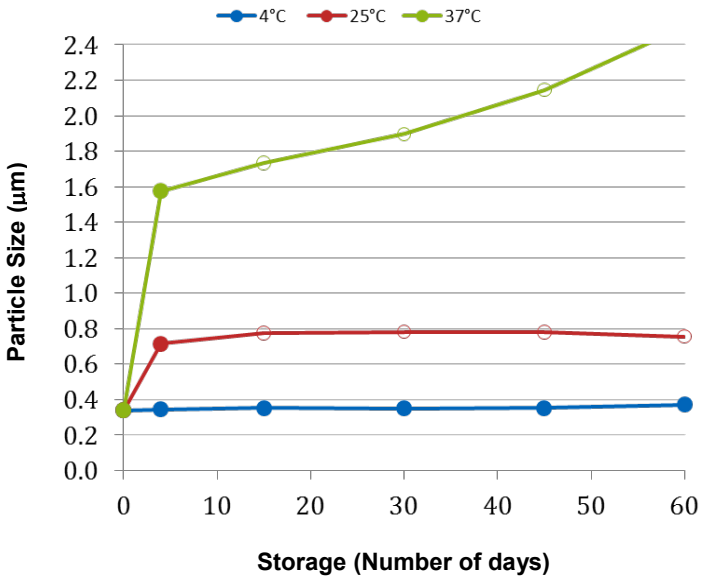


Figure 4: Effect of temperature on storage of solid lipid nanoparticles. The blue line (bottom) represents 4°C, the red line (second from bottom) represents 15°C and the green line (top) represents 37°C. The particle size of formulation stored at 37°C could not be measured (broken green line graph). Open green and red dots indicate that the particle size measurement is insignificant since the particle growth was evident after 4 days of storage.

Particle growth was examined over the storage period, and the particle size could not be measured after 60 days. Microviscosity (property of the emulsifier) prevents aggregation after particle contact and is dependent on temperature. Higher temperature reduces the microviscosity of the emulsifier and induces destabilisation of the system. Higher temperatures also increase the kinetic energy of the SLNs which is enough to overcome the electrostatic repulsion and form agglomerates.[24]

## 4. CONCLUSION

A Taguchi design enabled successful formulation of optimised solid lipid nanoparticle dispersions. A clear assessment of the importance of factors including the amount of stearic acid and the concentration of Tween®20 was provided by this analysis. Although this composition was the main factor influencing particle size and particle size distribution, it did not seem to affect the zeta potential. The addition of base or acid to alter the pH of the preparation before or after the process increased the particle size and zeta potential, and

hence the physical stability of the preparation. The addition of an electrolyte had a non-trivial impact upon the physical stability; however, an increase in particle size was evident.

The influence of co-emulsifiers and stabilisers in the development of finely dispersed nanoparticle dispersion was not fully evident since only the particle size distribution of nanoparticles was. However, the magnitude of the zeta potential was slightly reduced and the average particle size increased. Stability of the optimised formulation (75 mg stearic acid, 0.25% Tween®20 and 0.25% 0.1 M NaOH) was found to be higher under refrigerated conditions. In conclusion, initial experimental design and further optimisation of other formulation parameters have clearly shown their usefulness in understanding SLN formation and this study has constituted a framework for further research into SLNs as suitable carriers for chemotherapeutic agents.

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