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Scale-up of the preparation process of solid lipid nanospheres. Part I

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Abstract

An apparatus was designed to prepare solid lipid nanospheres (SLN), potential colloidal therapeutic system obtained by dispersing a warm oil-in-water (o/w) microemulsion in cold water. The apparatus, consisting mainly of a thermostated aluminium chamber and a pneumatic piston, permitted to disperse through a needle up to 100 ml of warm microemulsion and to vary the temperature, the dispersing rate and the drop size of the warm o/w microemulsion. Experimental design was applied to study the effect of four experimental factors, such as chamber temperature, piston pressure, needle gauge and volume of dispersing water, on average diameter and polydispersity index of SLN and on dispersing time of microemulsion (the time required for the microemulsion to drip completely from the apparatus). The results showed that temperature and pressure play the most important roles depending on the needle gauge used. In particular, the smallest SLN were obtained using high temperature and pressure values and a small needle gauge.

Keywords: Solid lipid nanospheres; Scale up; Experimental design; Fractional factorial design; Star design

1. Introduction

Numerous researchers have proposed solid lipid nanoparticles (SLN) as alternative colloidal therapeutic systems, using different preparative approaches (Schwartz et al., 1992; Westesen et al., 1993; Domb, 1995; Yang et al., 1999). Most of the SLN are prepared by high-pressure homogenisation of melted lipids.

SLN were prepared by the dispersion warm oil-in-water (o/w) microemulsions in cold water; solid lipids with low melting points were used as internal phase of the microemulsions (Gasco, 1997).

Considering the droplet structure as the structural organisation of o/w microemulsions, liquid oil nanodroplets are present in the warm o/w microemulsion; a rapid quenching of the warm

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o/w microemulsions in cold water permits the crystallisation of oil nanodroplets present in the microemulsion forming the solid nanospheres.

In previous research (Cavalli et al., 1996) a simple device to obtain reproducible SLN dispersions from warm o/w microemulsions was developed, consisting of a glass syringe thermostated by a bronze jacket. Its capacity was of 1 ml of microemulsion. The device provided precise control of the temperature and of delivery rate of the warm microemulsion, factors that affect the size of the SLN as well as the SLN composition. In order to evaluate the influence of the microemulsion components on SLN sizes three different microemulsion compositions were studied.

The present research aims to improve and scale up the device for the preparation of SLN; for this purpose an apparatus was designed permitting the dispersion in cold water of up to 100 ml of warm microemulsion for rapid production of larger amounts of SLNs. In the apparatus the warm liquid microemulsion is sterilizable through a 0.22 μm membrane filter as the nanodroplets sizes of warm o/w microemulsion are lower than 100 nm.

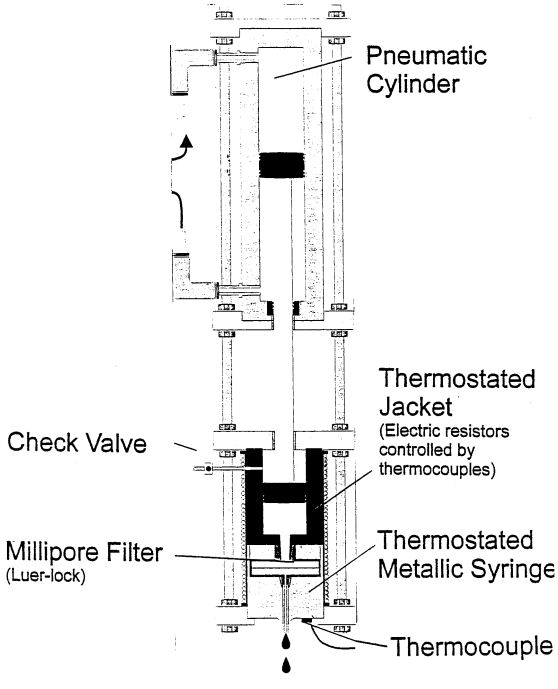

Fig. 1. Scheme of the apparatus.

To perform this study a previously optimised microemulsion formulation was used (Cavalli et al., 1997) following the test to verify if the microemulsion composition did not change increasing the amount of components.

The influence of different operating conditions of the apparatus on microemulsion dispersing time and SLN sizes was investigated using optimisation techniques. Using the apparatus, the optimal values of process parameters, such as pressure, temperature, needle gauge and volume of cold water in which the warm microemulsion is dispersed, were obtained.

2. Materials and methods

2.1. Materials

Stearic acid (m.p. = 68–70°C) was from Fluka (Buchs, CH); Epikuron 200 (soya phosphatidylcholine 95%) was a kind gift from Lucas Mayer (Hamburg, G); taurocholate sodium salt was a kind gift from PCA (Basaluzzo, I). Milli Q water was employed in all experiments.

2.2. Preparation of warm o/w microemulsion

SLN were prepared from a warm o/w microemulsion containing stearic acid as internal phase (0.70 mmol), Epikuron 200 as surfactant (0.14 mmol), taurocholate sodium salt as cosurfactant (0.66 mmol) and distilled water as continuous phase (111.11 mmol). To obtain the microemulsion, Epikuron 200 and warm water were added to the melted stearic acid at about 70°C. The cosurfactant was then added to the warm mixture, and a clear system was easily obtained under stirring at about 70°C. Batches of 50 ml of microemulsion were prepared; 25 ml of microemulsion in each experiment were employed.

2.3. Apparatus for the production of SLN

An apparatus was developed for the scaled-up preparation of SLN, the scheme of which is reported in Fig. 1.

The main part of the apparatus is a thermostated aluminium chamber (or syringe), holding up to 100 ml of microemulsion, in which a pneumatically operated piston supplies the microemulsion at a selected flux; at the bottom of the aluminum chamber there is a stainless steel support for a sterile membrane filter (0.22 μm) to guarantee the sterility of the product. The stainless steel support is connected to a needle by a Luer Lock connection. This apparatus is placed in an electric thermostated jacket from wich protrude only the needle for 3 mm.

After passing through the filter, the warm microemulsion flows through the interchangeable needle and drops directly into a stirred cold aqueous medium. A thermocouple measures the temperature of the aluminium chamber.

SLN were obtained by the following steps:

- the chamber is heated at the selected temperature;
- the chamber is filled with the warm o/w microemulsion previously formed;
- the chamber is connected to the filter support containing the 0.22 μm membrane;
- the chamber containing the microemulsion and the filter support are thermostated at the selected temperature;
- the warm microemulsion is dispersed under stirring into an ice-cooled capsule containing the right volume of water according to the microemulsion:water ratio selected; the water is stirred by a cylindrical magnetic bar at a fixed stirring rate (300 rpm). The microemulsion drips from the needle in the centre of the ice-cooled capsule; the needle is always placed at 4 cm from the water surface.
- the SLN dispersion is always stirred for further 15 min after the complete microemulsion dripping.

2.4. Experimental design

Experimental design (Box et al., 1978; Carlson, 1992) was used to optimize the preparation of SLN with the above mentioned apparatus and to evaluate the process factors mainly affecting the average diameter and the polydispersity index of SLN, in order to obtain the smallest SLN in a short time.

For this purpose the experimental parameters taken into account were:

- Pressure applied to the pneumatic cylinder (P)
- Temperature of the aluminium chamber (T)
- Needle gauge (N)
- Volume of the dispersing waters (DIL)

The choice of the needle gauge was limited by the needle availability on the market.

For each of the four experimental factors the lowest and highest admitted values defining the investigated experimental domain were selected. A fractional factorial design, or FFD, (Box et al., 1978; Carlson, 1992) was first performed (Table 1, experiments 1–8).

The responses chosen were average diameter (DIAM) and polydispersity index (POLI) of the SLN and dispersing time (TIME), the time required for the microemulsion to drip completely from the apparatus.

The experiments at 60°C were successively eliminated and substituted with experiments at the new temperature of 62°C because the temperature of 60°C proved to be critical for the microemulsion formation (Table 1, experiments 9–12).

The experimental design was augmented adding a Star Design (Deming and Morgan, 1987), to obtain a Composite Design (Khuri and Cornell, 1987), since a quadratic model appeared to be necessary from the FFD results analysis. The Star Design was replicated for the two needle gauge values (Table 1, experiments 13–24). All experiments were performed in triplicate.

The calculation of the regression models was performed on the range scaled (range ± 1) experimental factors values.

2.5. Characterisation of SLN

The average diameter and polydispersity index of SLN prepared using this apparatus were determined by photon correlation spectroscopy (PCS) using an N4 MD instrument (Coulter, USA) at a fixed angle of 90° and at a temperature of 25°C. Each value was the average of ten measurements. The polydispersity index is a measure of the size distribution of the nanoparticle population (Koppel, 1972). All SLN dispersions were diluted 1:40 with filtered water before analysis.

Table 1
The overview of parameters selected in the factorial design

Exp. no.	Pressure (bar)	Temperature (°C)	Needle gauge (mm)	Volume of water (ml)
1	0.5	60	0.3	50
2	1.5	60	0.3	200
3	0.5	70	0.3	200
4	1.5	70	0.3	50
5	0.5	60	0.6	200
6	1.5	60	0.6	50
7	0.5	70	0.6	50
8	1.5	70	0.6	200
9	0.5	62	0.3	50
10	1.5	62	0.3	200
11	0.5	62	0.6	200
12	1.5	62	0.6	50
13	1	62	0.3	125
14	1	62	0.6	125
15	1	72	0.3	125
16	1	72	0.6	125
17	1	65	0.3	20
18	1	65	0.6	20
19	1	65	0.3	230
20	1	65	0.6	230
21	0.5	65	0.3	125
22	0.5	65	0.6	125
23	1.7	65	0.3	125
24	1.7	65	0.6	125

2.6. Thermal analysis of water dispersion of SLN

Differential scanning calorimetry (DSC) analysis was performed using a DSC/7 differential scanning calorimeter equipped with a TAC 7/DX instrument controller TAC 7/DX. The instrument was calibrated with indium for melting point and heat of fusion. A heating rate of 20°C/min was employed in the 10–80°C temperature range. Standard aluminum sample pans (Perkin Elmer) were used to analyse the SLN dispersions; an empty pan was used as reference standard. Analyses were performed under nitrogen purge; triple runs were carried out on each sample.

2.7. Statistical analysis

A step-wise variable selection algorithm was used for optimal regression model evaluation. STATISTICA™ statistical package was used for the calculations.

3. Results

3.1. Experimental strategy

The experimental results obtained are reported in Table 2.

From the results of the first FFD (Table 2, experiments 1–8) a first order regression model with some second order interactions was calculated for each response. Because of the difference between the calculated and experimental result in the centre of the dominion, one decided to augment the design adding a Star Design, since a quadratic model appeared to be necessary. Since the needle gauge can assume only two fixed values, one decided to replicate the Star Design for both admitted values of the needle diameter (Table 2, experiments 13–24).

In this case the Star Design is not centered on the centre of the FFD. Moreover, due to operative constraints, such as temperature and pressure values, some of the Star Design extremes have

been shifted from their theoretical values. These changes do not affect significantly the final design that can still be used appropriately for the statistical analysis of the role played by the experimental factors.

3.2. Composite design data analysis

A regression model was calculated on the available experiments, applying a step-wise variable selection algorithm in order to choose the relevant variables involved. All the variables of a complete second order regression model were considered (experimental factors, their second order interactions and their squares).

No satisfactory regression model could be obtained for nanosphere diameter (best regression model: $R^2 = 0.5963$, four variables) and polydispersity index (best regression model: $R^2 = 0.3995$, two variables) using contemporary all the 20 experimental data.

Since there was evidence of different behaviour of the system with the two needle gauges, the data were separated according to the needle used (small or large). The regression models obtained for the two data sets are reported in two separate paragraphs.

3.3. Dispersing time

The regression model for the dispersing time, on the other side, was acceptable using all the experimental data together (best regression model: $R^2 = 0.9082$, five coefficients).

The variables selected by the step-wise variable selection algorithm, the corresponding regression coefficients and their standard errors are reported in Table 3.

The important role played by pressure is evident from the large regression coefficients of P and P^2.

The needle diameter, as could be expected, has a relevant effect as well. On the other hand it is

Table 2
Responses of fractional factorial design (FFD) and Star Design

Exp. no.	Average diameter (nm)	Polydispersity index	Disperding time (min)
1	75.0	0.78	92
2	36.7	0.14	36
3	66.5	0.76	80
4	27.1	0.12	22
5	62.5	0.41	44
6	36.5	0.17	8
7	34.5	0.40	78
8	30.1	0.19	18
9	68.0	0.63	90
10	36.7	0.14	36
11	60.5	0.45	42
12	36.5	0.17	8
13	42.7	0.28	35
14	43.3	0.27	38
15	38.0	0.20	30
16	41.0	0.18	27
17	55.6	0.35	40
18	54.3	0.13	35
19	56.0	0.24	36
20	36.0	0.20	35
21	37.0	0.18	80
22	40.7	0.25	64
23	38.0	0.15	13
24	35.0	0.22	11

Table 3
Best regression model coefficients and their standard error for TIME, 20 experiments, ($R^2 = 0.9082$)

Predictor	Coefficient	S.E.
Offset	32.73	2.39
P	−24.82	2.05
P^2	9.24	2.46
N	−5.64	1.66
P × DIL	7.98	2.74

difficult to explain the effect of dilution, whose interaction with pressure seems to be very large.

In Fig. 2, two 3-D contour-plots of time with respect to P and DIL, at constant needle diameter, are showed. These spline-plots show the global similarity of the two surfaces and confirm that the behaviour of the system changes slightly from small to large needle, with respect to TIME response.

3.4. Small needle data

3.4.1. Average diameter

The regression model refined by the step-wise variable selection algorithm contains the predictors reported in Table 4.

Its multivariate determination coefficient is 0.9624, which means that the regression model describes satisfactorily the experimental data.

The major role in the regression model is played by DIL. T and P are present in the regression model only as second order interactions. Temperature appears to be more important than pressure since its interactions have higher regression coefficients. DIL exerts a quadratic effect that can be explained by a visual inspection of the surface plot representing the experimental data and the spline model for the particle diameter as function of T and DIL (Fig. 3). The shape of this surface shows large diameters for two opposite experimental settings, i.e. small T, small DIL, or large T, large DIL. The best diameters, i.e. the smallest ones, can be obtained at high temperature and low dilution.

3.4.2. Polydispersity index

The regression model refined by the step-wise variable selection algorithm contains the predictors reported in Table 4. Its multivariate determination coefficient is 0.9646, extremely similar to the diameter one, which means that also this regression model describes satisfactorily the experimental data.

All the experimental factors exert relevant effect on polydispersity. The strongest effect seems to be due by the interaction between T and DIL. Both DIL and P show a quadratic effect. The dependence of POLI on the experimental factors is showed in the surface plots reported in Fig. 4. Also in this case the response is characterised by high values obtained for opposite experimental settings, i.e. low temperature, low dilution or high temperature, high dilution. The best polydispersity index, i.e. the smallest one, corresponds to high T, low DIL.

3.5. Large needle data

3.5.1. Average diameter

The regression model for the diameter, in the case of the large needle, is not very satisfactory. Its multivariate determination coefficient is only 0.5191. The regression model provides only information about the trend of the experimental data. The most relevant factors, are P and DIL (Table 5).

The spline surface plot of DIAM as function of P and DIL (Fig. 5) shows that in this case the best region is located at high P and DIL, while for other P values an average value of DIL seems preferable. The change to a large needle changes completely the pattern of functional dependency of DIAM on the experimental factors, in particular, T no longer plays an important role.

3.5.2. Polydispersity index

The regression model refined by the step-wise variable selection algorithm contains the predictors reported in Table 5. Its multivariate determination coefficient is 0.9600, which is similar to the results obtained for the small needle.

All the experimental factors exert an influence on polydispersity index. The dependencies are

showed graphically in the spline surface plots of Fig. 6.

The best experimental settings, leading to small polydispersity index, are high DIL and T or low DIL and T with average values of the pressure.

3.6. Model validation

The regression model were validated performing four further experiments, whose results were in agreement with the predicted ones (see Table 6).

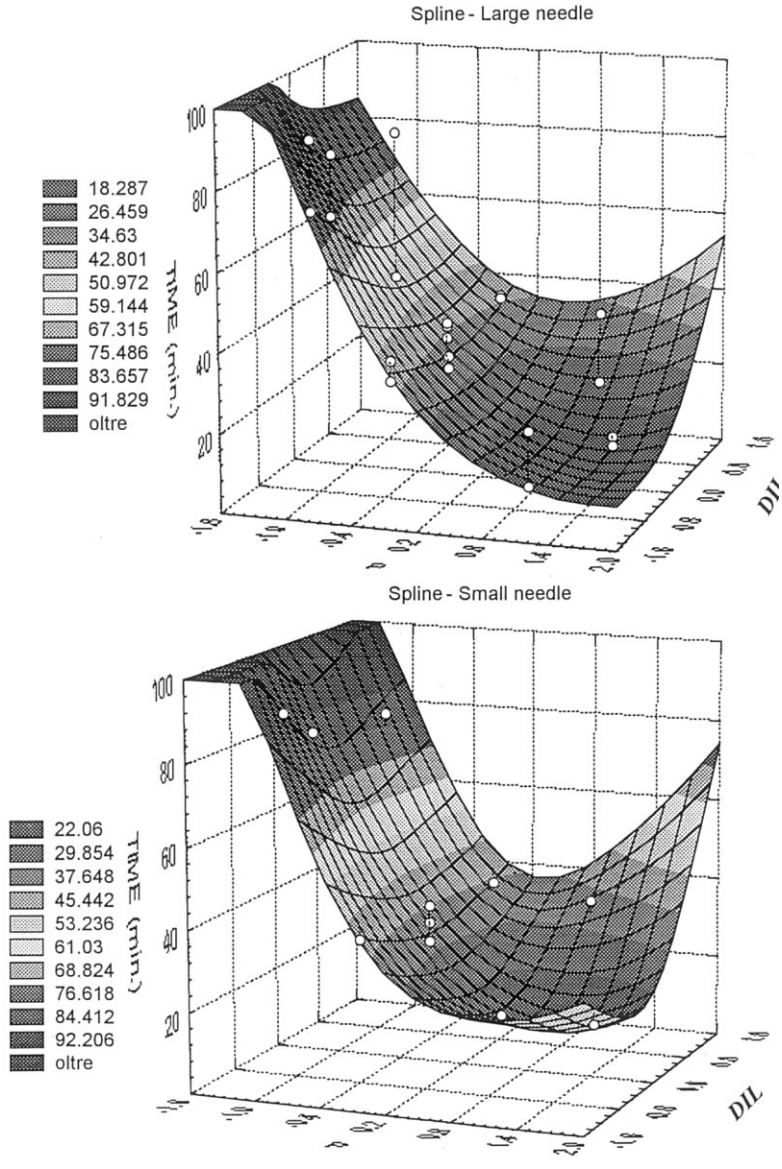

Fig. 2. 3D spline plots of TIME (min) versus P (bar) and DIL (ml) at constant needle gauge.

Table 4
Small needle: best regression models coefficients and their standard error for DIAM ($R^2 = 0.9624$) and POLI ($R^2 = 0.9646$)

Predictor	Coefficient for DIAM	Coefficient for POLI
Offset	37.71 (1.45)	0.235 (0.012)
T × DIL	15.11 (1.72)	0.111 (0.010)
DIL^2	9.26 (1.41)	0.033 (0.009)
P × DIL	4.52 (1.72)	–
DIL	4.18 (1.74)	–
P × T	3.69 (2.33)	–
P^2	–	−0.036 (0.009)
T	–	−0.019 (0.008)

3.7. Thermal analysis

The thermal analysis confirmed the crystalline state of SLN in the dispersions, being a melting peak at the melting temperature (69°C) of the stearic acid in the thermograms.

4. Discussion

The first approach to obtain an industrial production of SLN is to design step by step a scaled up apparatus for SLN preparation. Therefore the main aim of this research was to develop a first apparatus representing the first step of the scale up of the preparation process of SLN; the second aim was to study how some experimental factors influence the average diameter and polydispersity index of nanospheres and the dispersing time of microemulsion to achieve preliminary information for the complete scale up.

In this first part of the research the stirring rate of the dispersion was maintained fixed because previously it was shown that it did not influence the SLN sizes (Cavalli et al., 1996) and in other researches it was always maintained fixed.

In initial experiments the microemulsion/dispersing water ratio was varied from 1:1 to 1:10 (v/v); in this concentration range, SLN concentration does not affect SLN size. In the FFD experiments, the microemulsion/dispersing water ratios 1: 2 and 1:8 (v/v) were used, while in the Star Design experiments, the ranges of ratios was widened to 1:0.8 and 1:9.2.

The choice of the needle gauge is limited to 0.3 and 0.6 mm. to obtain microemulsion droplets not greater than 10 μl.

A previously studied o/w microemulsion formulation, consisting mainly of stearic acid and Epikuron 200, was chosen for data comparison

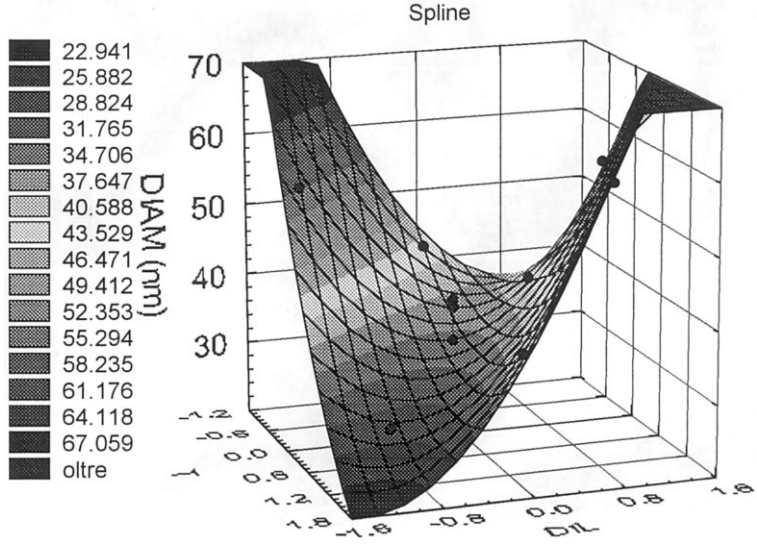

Fig. 3. 3D spline plot of DIAM (nm) vs. TIME (T, min) and DIL (ml).

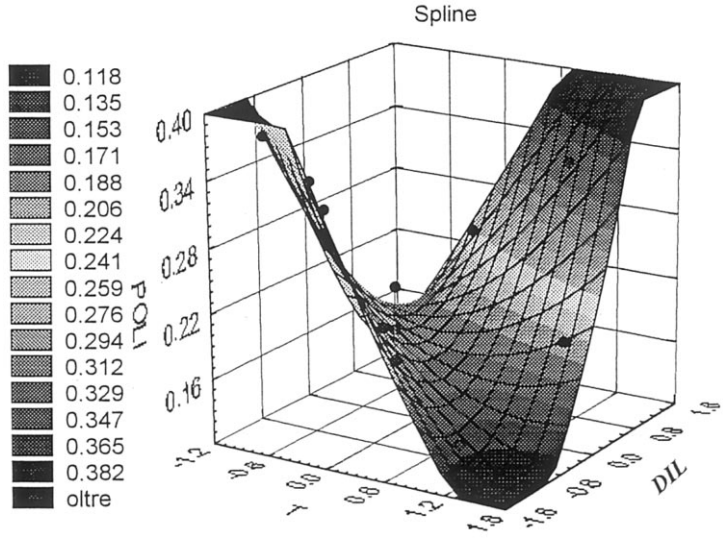

Fig. 4. Small needle 3D spline plot of POLI versus TIME (T, min) and DIL (ml).

purposes (Cavalli et al., 1997). The microemulsion is stable over time also when prepared in a quantity (100 ml) 35-fold greater than the previous one. In the previous work the o/w microemulsion was manually dispersed using a micropipette giving SLN with an average diameter of about 55 nm and a polydispersity index of about 0.2.

Thermal analysis of SLN dispersions of the present work showed the crystalline state of SLN.

From the statistical analysis the optimal conditions for dispersing time resulted high pressure (1.7 bar) and large needle (0.6 mm). Indeed the microemulsion dripped faster through the large needle than through the small one. Twenty five milliliters of microemulsion can be dispersed in 8 min while even 90 min were required for the same microemulsion volume with the smallest needle.

The regression models were validated performing four further experiments, whose results were in agreement with the predicted ones.

With the small needle, the most important experimental factors were DIL and T for average diameter; the polydispersity index was mainly affected by DIL and P. The regression models showed good correlations for both the responses,

Table 5
Large needle: best regression models coefficients and their standard error for DIAM ($R^2 = 0.5191$) and POLI ($R^2 = 0.9600$)

Predictor	Coefficient	S.E.
Offset	38.31 (3.31)	0.1512 (0.0160)
P	−5.96 (2.84)	–
P × DIL	−5.20 (3.90)	–
DIL^2	3.60 (3.21)	–
T × DIL	–	−0.0976 (0.0128)
P × T	–	0.0580 (010128)
P^2	–	0.0485 (0.0117)
T	–	−0.0522 (0.0109)
T^2	–	0.0645 (0.0137)

Table 6
Average diameter and polydispersity index of SLN and dispersing time obtained using the following experimental conditions: 1.0 bar, 65°C, 125 ml of water

	Average diameter (nm)	Polydispersity index	Dispersing time (min)
Small needle			
Value obtained	32.5	0.20	40
Value predicted	31.5	0.19	38
Large needle			
Value obtained	36.5	0.21	35
Value predicted	33.0	0.18	34

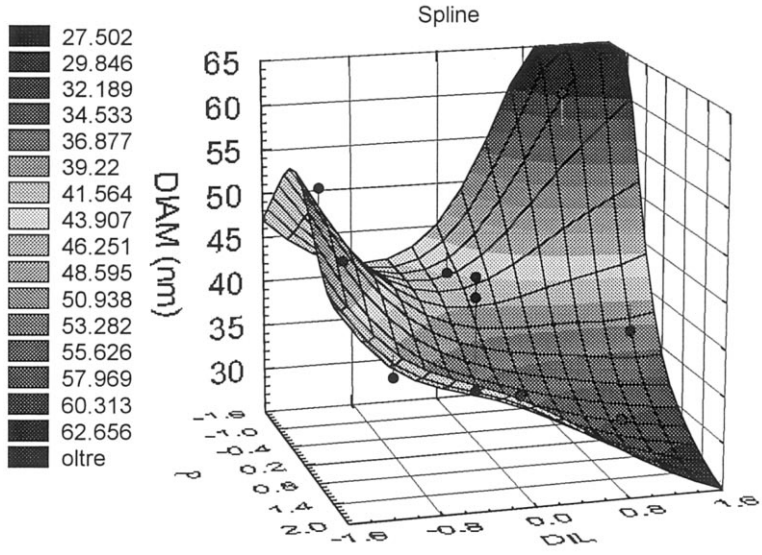

Fig. 5. Large needle: 3D spline plot of DIAM (nm) versus P (bar) and DIL (ml)

with a slightly sloping ridge along the diagonal direction from high T, low DIL to low T, high DIL. The optimal diameter is located along this direction. Indeed, the smallest average diameters and polydispersity indices were obtained using high T and small DIL values.

Using the large needle, the behaviour of the system is different; the main effects on SLN diameter are due to the factors P and DIL, but the regression coefficient is too small. The best experimental setting corresponds to high values of P and high values of DIL.

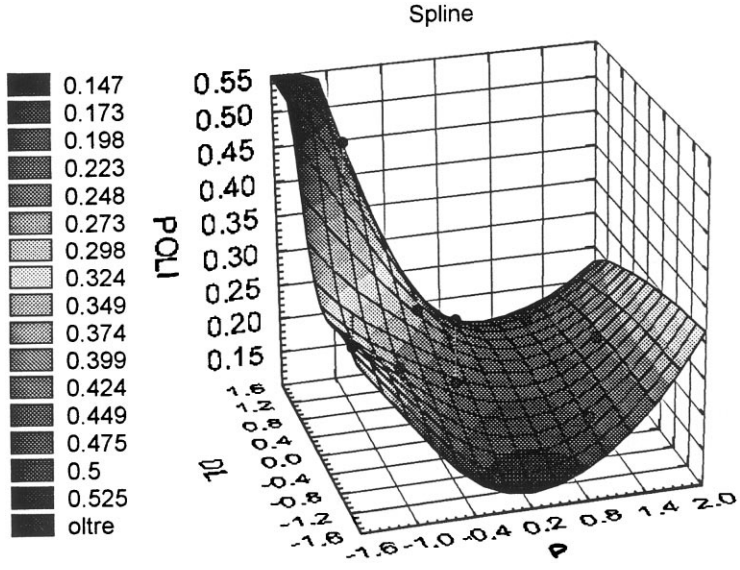

Fig. 6. Large needle: 3D spline plot of POLI versus P (bar) and DIL (ml).

The largest volume of dispersing water showed the best results (Fig. 5); a first hypothesis to explain this behaviour is that a lower volume of water might favour the aggregation of SLN, as the dripping rate of the microemulsion was higher with the large needle than with the small needle; the smaller volume of dispersing water may be not sufficient to completely disperse the microemulsion during the dispersion.

All experimental factors affected the polydispersity index with the large needle.

This different behaviour between the two needles used might be related to the preparation method of the SLN. SLN are formed by rapid quenching of the warm o/w microemulsion and, as previously shown (Cavalli et al., 1996), the temperature difference between the warm microemulsion and the cold dispersing water plays an important role on the size of SLN. A rapid crystallisation of the oil droplets of the warm microemulsion during quenching favours the formation of small SLNs, avoiding the coalescence among the oil nanodroplets.

In the same conditions of P, the warm microemulsion dripped more slowly and with smaller volumes through the small needle than through the larger one. The temperature (T) of the aluminium chamber of the apparatus, corresponding to the temperature of the warm o/w microemulsion, is a factor that affected the SLN size. The greatest SLN sizes were obtained when the lower values of T were used.

In conclusion, the SLN preparation process was successfully scaled-up with the above-mentioned apparatus; it showed an easy performance and permitted to obtain reproducible SLN dispersions.

Modifying the operating parameters, SLN of different sizes were obtained.

The optimal process conditions were determined for each of the needles used. From experimental design it was shown that the best performance can be obtained using the small needle. Indeed, using the small needle, with high temperature and pressure values, SLN with an average diameter of about 26 nm and a polydispersity index of 0.1 was obtained.

Research is in progress to reach the second step of the scale up design. The apparatus will be improved with a multiple needle system, a hopper where prepare the warm o/w microemulsion and connected to the aluminium chamber. A system controlling the temperature of the dispersing water, the stirrer form and size with the stirring rate will be also studied.

Acknowledgements

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