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Research Article

The Effects of Supercritical Carbon Dioxide Processing on Progesterone Dispersion Systems: a Multivariate Study

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Abstract. The aim of this work was to investigate the effects of supercritical carbon dioxide (SC-CO_2) processing on the release profiles of progesterone (PGN) and Gelucire 44/14 dispersion systems. A fractional factorial design was conducted for optimization of the particles from gas-saturated suspension (PGSS) method and formulation parameters and evaluating the effects of three independent responses: PGSS process yield, *in vitro* dissolution extent after 20 min (E_{20}) and $t_{1/2}$ for prepared PGN dispersion systems. The experimental domain included seven factors measured at two levels to determine which factors represent the greatest amount of variation, hence the most influence on the resulting PGN dispersion systems. Variables tested were temperature (*A*) and pressure (*B*) of the supercritical fluid, sample loading (*C*), SC-CO_2 processing time (*D*), sonication (*E*), drug-to-excipient ratio (*F*) and orifice diameter into the expansion chamber (*G*). The analysis of variance showed that the factors tested had significant effects on the responses (p value <0.05). It was found that the optimum values of the PGSS process are higher pressure (186 bar), higher temperature (60°C), a longer processing time (30 min) and lower PGN-to-excipient ratio of 1:10. The corresponding processing yield was 94.7%, extent of PGN dissolution after 20 min was 85.6% and the $t_{1/2}$ was 17.7 min. The results suggest that Gelucire 44/14-based dispersion systems might represent a promising formulation for delivery of PGN. The preparation of PGN-loaded Gelucire 44/14 dispersion systems from a PGSS method can be optimized by factorial design experimentation.

KEY WORDS: factorial design experiment; *in vitro* dissolution; optimization; particles from gas-saturated suspensions (PGSS); process yield.

INTRODUCTION

Over the last few decades, supercritical fluid (SCF) techniques have emerged in the pharmaceutical industry showing promising results for different applications including extraction, drug particle size reduction, and mixing (1–4). A SCF shows greater diffusivity than its corresponding liquid, leading to the improved uniformity of a solid dispersion. Molecular dispersions are also possible, but such dispersions are a function of a drug and its excipient solubility in a SCF (5). Carbon dioxide (CO_2) is the most commonly used agent because it is non-toxic (GRASS), readily available and inexpensive (1). Furthermore, supercritical carbon dioxide (SC-CO_2) has a low critical point for temperature (31.1°C) and pressure (74 bar), making it practical for heat-sensitive drugs (1,2).

A limitation of SCF is that, although it provides high speed results, it is difficult to know if it has been used in the most effective way. The typical SCF testing conditions seem to have a heuristic element. In other words, there are no set guides in the construction of a SCF unit and there are no standardized operating procedures to follow, leading to numerous experimental conditions that could be utilized. This could be due to the fact that SCF technology is a relatively new technique in the pharmaceutical sciences (6). The use of a screening experiment could solve the problems of production efficiency by signalling important parameters early in the research phase. With the important parameters defined and the unimportant parameters discarded, a formulation can be optimized using a combination of the most important parameters.

A relatively uncommon SCF method, as used in this study, is the particles from gas-saturated suspension (PGSS). The PGSS method involves compressing a gas into a SCF (processing both liquid and gas properties) and dispersing and/or dissolving constituents throughout this fluid to form a suspension or solution, which is then rapidly expanded into a collection chamber under ambient conditions, resulting in the

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evaporation of the gas and formation of solid or semi-solid dispersion systems (5). The form of dispersion depends on the physicochemical properties of the constituents, for example, higher molecular weight PEGs form solid dispersions, while lower molecular weight PEGs form suspensions and a mixture of high and low molecular weight PEGs form semi-solid dispersions (7). It was thought that SCF-based technology may offer unique opportunities in the formation of progesterone (PGN) dispersion systems and provide an alternative method to some of the conventional methods available such as co-melting and use of co-solvents. Since this SCF method is less frequently used, especially as a formulation method for transdermal delivery, it is perhaps one of the least researched and its parameters are amongst the most ambiguous. For example, in order to reduce the particle size of a drug by rapid expansion of the supercritical solution (RESS) method, the use of a nozzle during the SCF expansion phase is essential to break-up the expanding solution into solid particles (8–10). However, for the PGSS method where mixing efficiency is of utmost importance, the use of a nozzle may or may not be required. In addition, the RESS or gas anti-solvent (GAS) methods are dependent on the solvent or anti-solvent function of the SCF in order to produce products with desired properties (11). However, questions remain regarding the PGSS method and solubility capacity and whether or not it is critical to the process of mixing constituents into an optimal formulation. For example, it is known that some polymers that can draw up high amounts of SC-CO_2 (10–40%) rather than dissolve in the SCF because the polymer either swells or melts at a SCF temperature (>32°C) which may occur below its normal melting/glass transition temperature (12–15). During the expansion phase, the liquid cools below its solidification temperature, due to the Joule–Thompson effect (16), evaporation and volume expansion of the gas, producing solid or semi-solid particles dispersed throughout solid or semi-solid excipient(s)/carrier systems (3). Hence, unlike other SCF techniques, PGSS methods do not necessarily require materials to be soluble in the SC-CO_2 (5). Furthermore, one of the pivotal parameters is the melting profile of a drug and excipient in the presence of SC-CO_2, thus knowledge of the pressure–temperature trace and solid–liquid–vapour and liquid–SCF–vapour equilibrium is required to give the ideal pressure needed to melt and form an ideal SCF at a given temperature (17–19). Based on this knowledge, a set of tables could be developed and used as a reference to guide research towards an optimized SCF process and enhanced drug formulation. In this study, a set of parameters was investigated to help define possible critical factors, which could influence the formation of PGN-loaded Gelucire 44/14 dispersion systems. A schematic of the SCF unit as used in this study has been shown in Fig. 1. Following is an outline of the procedure used with each of the steps that were involved in the PGSS method using a SCF unit.

PGSS Processing

As described in some detail earlier, the PGSS process consists of three steps: preparing the drug and excipient, SC-CO_2 processing which involves forming the supercritical mixture and rapid expansion to non-SCF conditions and recovery of the final product. This can be further broken down into three categories that describe the procedure: preparation, processing and recovery. Each step is important and has the potential to influence the final product.

An important step prior to the preparation of SCF processing involves the selection of the excipients to be used to incorporate and carry a drug. Lipid-based amphiphilic excipients has been used to some extent in SCF research, such as Gelucire 44/14 and d-α-tocopheryl polyethylene glycol 1000 succinate (TPGS) (2). Various studies that have found improved dissolution and oral absorption using Gelucire 44/14 and SCF methods (2,7,20,21). Based on this information and the readily availability of Gelucire 44/14, it was selected as the model excipient in preparation of PGN dispersion systems using SCF processing. Gelucire 44/14 is a saturated polyglycolized glyceride made up of a defined combination of mono-, di- and triglycerides, mono- and di-fatty acid esters of PEG 1500 and free PEG 1500 (2,22,23). The hydrophilic–lipophilic balance is 14 and melting point are relatively low at 44°C (2,7). The critical micelle concentration of 0.1% *w*/*w* of Gelucire 44/14 means that solubilising lipophilic drugs once in an aqueous environment is a possible advantage.

The main aim of this study was to investigate the effect of several experimental parameters on the SCF processing of PGN with the excipient Gelucire 44/14 and secondly to find the best experimental conditions to form an optimized PGN dispersion systems. The study outlined in this paper employed a symmetrical fractional factorial design at two levels to investigate variables identified in the PGN dispersion process. This was expressed as 2^{k-p}, where k is the number of variables and p, or generators, are the number of columns in the experimental domain constructed from a full factorial design 2^{β} ($\beta=k-p$) (24,25). The variables measured were pressure (A), temperature (B), sample loading (C), carbon dioxide processing time (D), sonication (E), PGN/excipient ratio (F) and orifice diameter into the expansion chamber (G). This design was used to determine the effects of the seven variable parameters expected to have an effect on the performance of the SCF-based PGSS method evaluated by three responses. The responses measured were process yield, *in vitro* dissolution after 20 min (E_{20}) and $t_{1/2}$ of the PGN dispersion systems formed from SCF processing with CO_2.

A fractional factorial study is an effective means to identify principal variables with minimal resources. The use of a fractional factorial design, when compared to a full factorial design, reduces the number of experimental runs from 128 to 16. This resolution IV factorial study identified the main effects and first-order interactions between the seven factors investigated. So far, there were no similar multivariate studies previously applied to the optimization of SCF processing of PGN in various excipients. This study will hopefully provide a great deal of information about the behaviour of the ternary system (SCF/PGN/excipient) by studying the interactions among the variables and the modelling of multifactorial responses based on a relatively small number of runs.

EXPERIMENTAL

Materials

Industrial grade PGN was purchased from Pfizer and Pharmacia Company (New York, USA). Gelucire 44/14 was

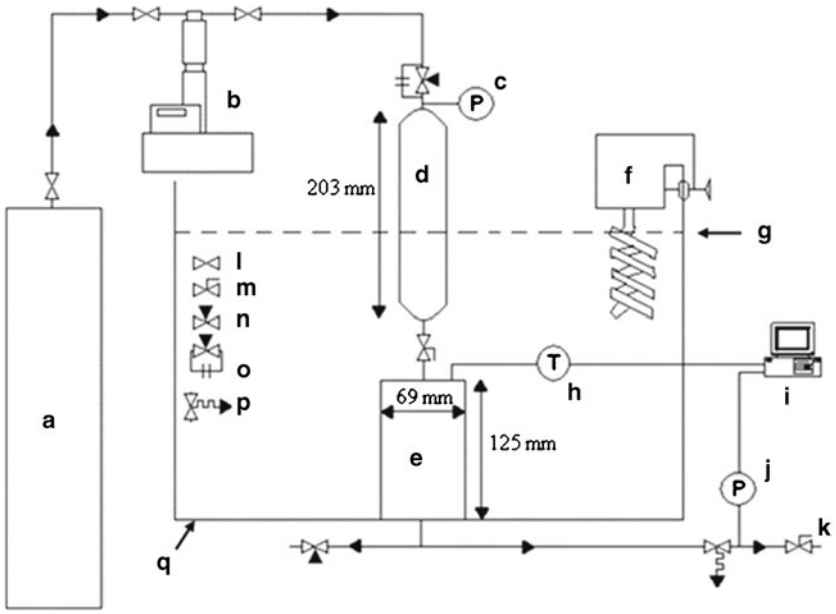

Fig. 1. A schematic of the SCF unit used to perform the PGSS method. *a* Liquid CO_2 cylinder, *b* syringe pump, *c* pressure gauge, *d* sample cylinder, *e* precipitation chamber, *f* heat pump, *g* water line, *h* temperature transmitter, *i* computer with PicoLog software, *j* pressure transducer, *k* CO_2 vapour (outlet), in *line arrows* indicate flow of CO_2, *l* isolation valve, *m* ball valve, *n* needle valve, *o* needle valve with rupture disc, *p* relief valve, and *q* water bath. Not drawn to scale

donated by Gattefossé Corporation (New Jersey, USA). Methanol and acetonitrile was high-performance liquid chromatography (HPLC) grade, which was purchased from Sigma-Aldrich (Auckland, New Zealand). Liquid CO_2 (purity 98%) was purchased from BOC Gas (Auckland, New Zealand). Triple-distilled water was obtained in-house by reverse osmosis (Milli-Q, Millipore, USA). All samples were used without any further purification steps.

SCF Processing and Experimental Domain

The dispersion systems were formed using an in-house purpose built SCF unit capable of performing the particles from PGSS method. Briefly, as described earlier, liquid CO_2 was pumped into a sample cylinder (Swagelok, Solon, USA) containing the drug PGN powder and Gelucire 44/14 of desired weight (3, 6 or 9 g) and ratio (1:1, 1:5 or 1:10). Seven variables were identified as possible critical points in the dispersion processed by SCF treatment. The materials were then dispersed under the designed conditions predetermined in the experimental domain, see Table I. After each condition was met, the SCF dispersion was released into the attached expansion chamber at atmospheric pressure and room temperature. At the end of the dispersion process, the dry semi-solid mass was removed and stored in glass vials for later analysis.

Formation of PGN dispersion systems using SCF was mainly governed by the density of the fluid, and therefore, pressure (bar) and temperature (degrees Celsius) were expected to be very important factors. The pressure and temperature factors and the other five selected factors and their corresponding ranges (+1, upper, and −1, lower levels) were determined after preliminary screening experiments. Two centre point runs (0) were also included, set at a medium level between the upper and lower levels. All the pressure levels tested were in the supercritical region for CO_2, while several runs had temperatures below the SC-CO_2 level of approximately 31°C (*i.e.* there were some sub-critical experimental runs investigated). The temperature range was decided to be a lower level of 20°C and upper level of 60°C, thus the centre was 40°C, which is similar to the melting point of Gelucire 44/14.

The duplication of centre point was used to estimate the experimental error. All experiments were randomly performed without replication. The measured responses were defined as the percent process yield, *in vitro* dissolution (extent) after 20 min and $t_{1/2}$.

Experimental Matrix

The factorial design of 2^{7-3} was used to give 16 experimental runs, instead of the 128 runs required for a full 2^7 factorial design. The experimental matrix is provided in Table II together with the corresponding responses for Y_1, Y_2 and Y_3. The recurring result between the centre points

Table I. SCF Processing and Experimental Domain

Factor		Low level (−1)	Centre point (0)	High level (+1)
A	Pressure (bar)	90	135	186
B	Temperature (°C)	20	40	60
C	Sample load (g)	3	6	9
D	Processing time	15	22	30
E	Sonication (min)	0	5	10
F	Drug/excipient ratio	1:1	1:5	1:10
G	Orifice diameter (in.)	Small (1/16)	Medium (1/8)	Large (1/4)

Table II. Experimental Matrix for the 2^{7-3} Design and Responses for PGN Dispersions

Run	SCF processing conditions						
	Factors						
	A	*B*	*C*	*D*	*E*	*F*	*G*
X1	1	−1	1	1	−1	1	1
X2	1	1	−1	−1	−1	1	1
X3	**0**	**0**	**0**	**0**	**0**	**0**	**0**
X4	−1	−1	−1	−1	−1	−1	−1
X5	−1	1	−1	1	1	−1	1
X6	1	−1	−1	1	1	1	−1
X7	−1	−1	1	−1	1	1	1
X8	−1	1	1	1	−1	1	−1
X9	1	1	1	−1	1	−1	−1
X10	−1	1	−1	−1	1	1	−1
X11	**0**	**0**	**0**	**0**	**0**	**0**	**0**
X12	1	1	1	1	1	1	1
X13	1	−1	−1	−1	1	−1	1
X14	1	1	−1	1	−1	−1	−1
X15	1	−1	1	−1	−1	1	−1
X16	−1	−1	1	1	1	−1	−1
X17	−1	1	1	−1	−1	−1	1
X18	−1	−1	−1	1	−1	1	1

Rows in bold are the centre points
SCF = supercritical fluid, A = pressure, B = temperature, C = sample load, D = contact time with CO_2, E = sonication, F = drug/excipient ratio, G = orifice diameter

(runs 3 and 11) for every response implies that the SCF processing was very reproducible.

Resolution

The factors *A* to *D* on the experimental matrix were from a full 2^4 factorial design. Factors E, F and G are the generators which were formed by multiplying the previous four columns. The *shortest word* in the generated design was 4 units, providing a resolution IV experimental design, that is $E = A{\cdot}B{\cdot}C$, $F = B{\cdot}C{\cdot}D$ and $G = A{\cdot}C{\cdot}D$, and the *generating relations* can be expressed as:

$$[I = ABCE, I = BCDF, I = ACDG].$$

Regression Modelling

Multiple regression gives a mathematical relationship between responses and independent variables (26). A fractional factorial design provides sufficient data to fit a linear regression, as given below for seven factors (coded values):

$$Y = b_0 + b_1A + b_2B + b_3C + b_4D + \ldots + b_{14}BD + b_{15}ABD + \varepsilon \quad (1)$$

where *Y* represents the response, b_0 the intercept, b_i the parametric coefficients of the model obtained by regression, *A*, *B*, *C* and *D* are the independent experimental factors (coded variables) and ε is the error term derived from the centre points.

PGN Recovery

In order to ensure that the test samples contained PGN within 25% *w*/*w* of the original amount of PGN loaded into the sample cylinder, the resulting products after SCF processing were analysed by HPLC for the amount of PGN recovered. Samples were tested in triplicate.

Percentage Process Yield

The yield of the collected dispersion systems by SCF processing was calculated by weight measurements as the amount of the produced sample in the precipitation chamber divided by the amount of mass initially introduced in the sample cylinder. The process yield was expressed as a percentage. This was used as a response to evaluate the selected variables and optimize the SCF process for the best possible yield.

In Vitro Dissolution

Samples were removed from the filter located at the base of the expansion chamber (PGSS unit). The recovered samples were weighted to 4±0.2 mg and used in the dissolution studies. The *in vitro* dissolution studies of the PGN dispersion systems were carried out in vessels containing 900 mL Milli-Q water and stirring with a paddle at 100 rpm rotated by a USP apparatus 5 (Hanson Research, SR8PLUS dissolution apparatus, California, USA) at 37°C. Each PGN dissolution was carried out in triplicate. The dissolution profiles were used to determine two responses: PGN dissolution extent after 20 min (E_{20}) and time to dissolve 50% of the dispersed PGN-loaded Gelucire 44/14 ($t_{1/2}$). These responses were used to evaluate the effects of SCF processing on PGN-loaded Gelucire 44/14 and optimize the dispersion based on the aqueous dissolution of PGN.

Statistical Analysis

After dissolution, the estimated amount of PGN released from the dispersion systems was compared to each other using Eqs. 1, 2 and 3. The data were evaluated using the factorial design function from the Minitab® Release 15.0 software (Minitab Inc. State College, Pennsylvania, USA). Statistical analyses were performed by analysis of variance (ANOVA) and regression coefficients were calculated. Statistical significance was defined as p value <0.05. Fisher's F test was conducted to test the adequacy of the model.

RESULTS

Recovery of PGN

Representative chromatographic profiles for PGN and PGN-loaded Gelucire 44/14 are shown in Fig. 2. The recovery of PGN from the samples was between −77.04% and 106.95%, indicating large variability of uniformity for the SCF process. However, recovery of PGN within each sample was less variable, suggesting that control over PGN recovery is possible under each set of conditions tested (shown in Table III).

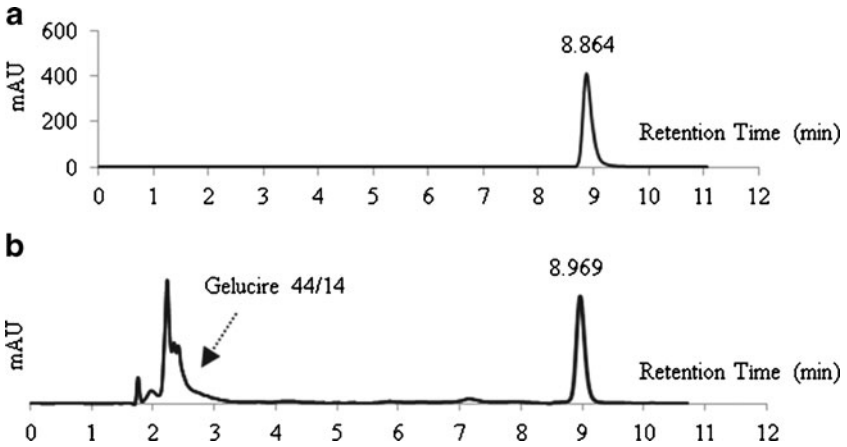

Fig. 2. Representative chromatograms showing **a** standard PGN of 100 μgmL^{-1} and **b** PGN-loaded Gelucire 44/14

Evaluation of Factorial Design

Figure 3 shows the release profiles of PGN from Gelucire 44/14 of different ratios; identification of the analyte extracted from the matrix was performed by injecting standard solutions into HPLC with UV–vis detection (DAD). As various parameters potentially affect the PGSS process, the optimization of the experimental conditions represents a critical step in the development of a SCF method (1). It is known, for example, that solubility of the analyte can be controlled by the composition, density and temperature of the SCF (1,27). Moreover, the recovered product is not only dependent on the operating conditions but also on the sample characteristics, *e.g.* water content, matrix type, particle size, viscosity, *etc.*, making selection of optimum conditions difficult, especially with subsequent reliable quantification (1,4,5,28–30). Furthermore, on the basis of preliminary experiments and the literature, some experimental parameters were not varied or examined, *e.g.* continuous CO_2 flow (2,31), the collection filter (300-μm stainless steel slab) (12), multiple SC fluids (32), use of a nozzle (3,32,33), the use of a modifier (ethanol) (1,12) and the release rate into the precipitation chamber (12).

Table III. PGN Recovery/Drug Loading After SCF Processing, $n=3$

Sample	Mean PGN Recovery (%)±SD
X1	115.00±2.24
X2	105.77±0.65
X3	**100.19±0.037**
X4	77.04±3.56
X5	106.95±1.54
X6	106.40±1.09
X7	99.04±0.15
X8	103.90±2.04
X9	102.77±1.94
X10	98.75±0.56
X11	**98.28±0.62**
X12	99.00±0.49
X13	103.09±1.86
X14	102.03±1.23
X15	104.08±1.29
X16	99.56±0.097
X17	101.53±1.34
X18	103.04±1.78

Rows in bold are the centre points
SD = standard deviation, PGN = progesterone

The main effects considered have not all shown obvious correlations with the responses tested, which may mean there were some varibles not examined that had an effect or too many varibles were examined for a given response. The main effects of the variables sample laod (C), sonication time (E), drug/excipient ratio (F) and diametre orifice (G) were not significant for all the responses, whereas main effects of the variables pressure (A), temperature (B) and CO_2 contact time (D) resulted to be significant. In addition, interaction effects of the variables (A) and (F) were significant for all the responses except that for $t_{1/2}$. On the basis of the results of the factorial design obtained for $t_{1/2}$, the variable (F) could have been excluded from the experiment because it was not significant either as main and interaction effect. No square terms appear in the polynomial functions for any of the responses investigated, indicating that the main effects and interactions have occurred due to the influence of the factors examined and not any independent variables

Y_1—Process Yield

Results for the Y_1 study were analysed for the effect, coefficients, standard error of the coefficients, T values, and p values are shown in Table IV. Regression for this response was excellent at 99.97% and four factors showed a significant effect and one interaction between two factors was also significant (p value <0.05).

The data showed that the pressure (A), temperature (B), processing time (D) and PGN/excipient ratio (F) were significant main effects influencing the yield of PGN dispersion systems manufactured using SC-CO_2 processing ranging between 2.4% and 94.7% yield (p value <0.05). By substituting the regression coefficients in Table IV, a mathematical model can be obtained to estimate the percentage yield of PGN processing with SC-CO_2. Using this model and the Student's t test results and

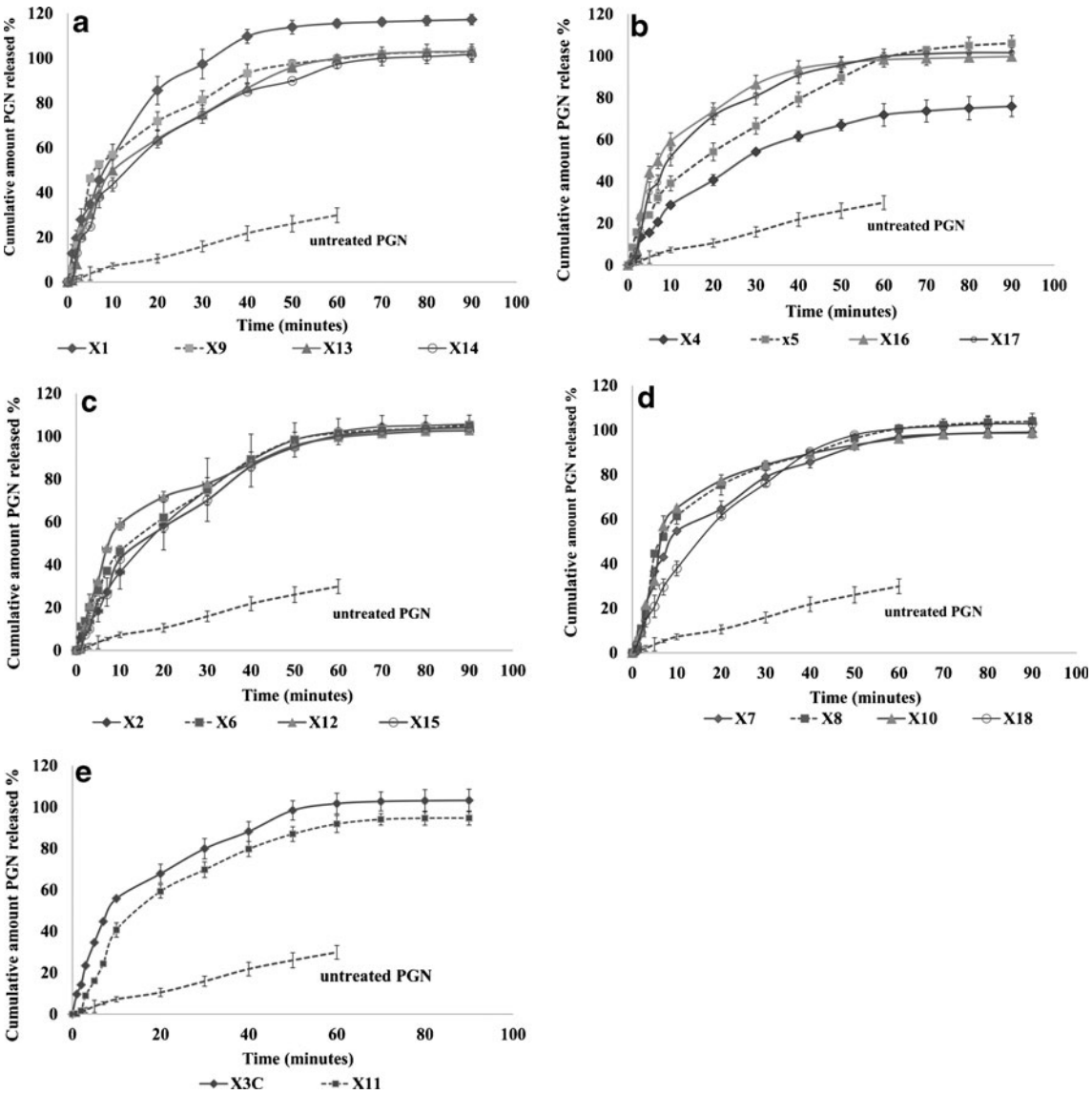

Fig. 3. Release profiles of the dispersion systems containing PGN are shown in: **a** PGN release from Gelucire 44/14 1:1 drug/excipient ratio (high pressure, 186 bar); **b** PGN release from Gelucire 44/14 1:1 drug/excipient ratio (low pressure, 90 bar); **c** PGN release from Gelucire 44/14 1:10 drug/excipient ratio (high pressure, 186 bar); **d** PGN release from Gelucire 44/14 1:10 drug/excipient ratio (low pressure, 90 bar); **e** PGN release from Gelucire 44/14 1:5 drug/excipient ratio (medium pressure, 135 bar)

the values in Table V, a simplified model was formed as expressed by Eq. 2:

$$Y_1 = 61.913 + 12.012A + 11.987B + 11.175D + 10.375F - 6.125AF. \quad (2)$$

Table V shows what the estimated yields are compared between the two models and the observed values in the factorial study. The use of the full mathematical model and the simplified model (Eq. 2) produced relatively accurate estimations of the observed processing yield with mean standard errors of 1.7 and 3.1, respectively. This model was able to show that for processing yield was independent of the insignificant factors, which were the sample load (C), sonication (E) and orifice diameter (G). The data show that the use of higher pressure (A) of 186 bar, higher temperature (B) of 60°C, a longer processing time (D) of 30 min and lower PGN-to-excipient ratio (Gelucire 44/14) (F) of 1:10 were all important features to the increasing yield of PGN dispersion systems using the PGSS method.

As described earlier, multiple interactions in this factorial design study do not provide any valuable information due to confounding. This means that it is uncertain whether the response was a result of the main factor effect or joint effects from the confounding interactions. The interactions illustrated the effects of two-factor interactions on the processing yield of PGN dispersion systems. The only combination that was able to be compared without be confounded was pressure (A) and PGN/excipient ratio (F). The interaction result shows that a significant increase in yield occurs when a high pressure (186 bar) was used in combination with lower PGN amount to Gelucire 44/14 (1:10) (p value=0.044). A higher

Table IV. Estimated Effects and Coefficients for Processing Yield (R^2=99.97%)

Term	Effect	Coefficient	SE coefficient	*T* value	*p* value
Constant	–	61.913	0.4250	145.68	0.004
A	24.025	12.012	0.4250	28.26	0.023*
B	23.975	11.987	0.4250	28.21	0.023*
C	−3.925	−1.962	0.4250	−4.62	0.136
D	22.350	11.175	0.4250	26.29	0.024*
E	4.175	2.088	0.4250	4.91	0.128
F	20.750	10.375	0.4250	24.41	0.026*
G	5.900	2.950	0.4250	6.94	0.091
AB	−8.125	−4.063	0.4250	−9.56	0.066
AC	−1.425	−0.713	0.4250	−1.68	0.342
AD	−3.650	−1.825	0.4250	−4.29	0.146
AE	−2.525	−1.263	0.4250	−2.97	0.207
AF	−12.250	−6.125	0.4250	−14.41	0.044*
AG	−4.700	−2.350	0.4250	−5.53	0.114
BD	5.950	2.975	0.4250	7.00	0.090
Centre points	–	−0.412	1.2750	−0.32	0.801

A = pressure, B = temperature, C = sample load, D = contact time with CO_2, E = sonication, F = drug/excipient ratio, G = orifice diameter, SE = standard error
*p value ≤0.05, statistically significant variables (ANOVA)

Table VI. Estimated Effects and Coefficients for Dissolution After 20 min (R^2=99.99%)

Term	Effect	Coefficient	SE coefficient	*T* value	*p* value
Constant	–	67.030	0.08938	749.91	0.001
A	4.465	2.232	0.08938	24.98	0.025*
B	6.720	3.360	0.08938	37.59	0.017*
C	13.777	6.889	0.08938	77.07	0.008*
D	2.613	1.306	0.08938	14.62	0.043*
E	5.705	2.853	0.08938	31.91	0.020*
F	−1.898	−0.949	0.08938	−10.62	0.060
G	−1.266	−0.633	0.08938	−7.08	0.089
AB	−2.764	−1.382	0.08938	−15.46	0.041*
AC	1.017	0.509	0.08938	5.69	0.111
AD	−0.014	−0.007	0.08938	−0.08	0.950
AE	0.506	0.253	0.08938	2.83	0.216
AF	−11.730	−5.865	0.08938	−65.62	0.010*
AG	2.573	1.286	0.08938	14.39	0.044*
BD	−11.321	−5.660	0.08938	−63.33	0.010*
Centre points	–	−3.475	0.26815	−12.96	0.049

A = pressure, B = temperature, C = sample load, D = contact time with CO_2, E = sonication, F = drug/excipient ratio, G = orifice diameter
*p value ≤0.05, statistically significant variables (ANOVA)

temperature (60°C) was also deemed to be important (p value =0.023), along with the use of a longer processing time (p value=0.024), as well as a lower PGN-to-excipient ratio (p value=0.026) to produce the maximum yield of PGN dispersion systems from the PGSS unit using SC-CO_2.

Y_2—Extent of PGN Dissolution After 20 min

The statistical parameters for the extent of PGN dissolution after 20 min are shown in Table VI. Table VI shows that the significant factors gave results ranging between 40.7% and 85.6% PGN dissolved after only 20 min. All of the investigated variables in the SC-CO_2 processing of PGN dispersion systems, except for PGN/excipient ratio (F) and orifice diameter (G), had a significant influence on the dissolution of PGN over 20 min. Of all the variables, the sample loading (C) had the greatest effect on PGN dissolution over 20 min (response Y_3), followed by temperature (B), then pressure (A) and lastly processing time (D). The PGN dissolution over the initial 20 min for PGN dispersion systems manufactured using a low sample load (40.7–77.4%) was lower than the range of extent dissolved using a high sample load (57.6–85.6%).

Table V. Comparison of Observed and Estimated Processing Yields from the Full and Simplified Model (Eq. 3)

Run	Observed yield %	Estimated yield % (full model)	SE	Estimated yield % (simplified model)	SE
1	67.701	67.5583	1.6941	71.1958	3.0653
2	78.706	78.5583	1.6941	81.3208	3.0653
3	62.709	63.8333	1.2671	63.8333	3.8897
4	2.415	2.2583	1.6941	2.5708	3.0653
5	77.867	77.6583	1.6941	68.8958	3.0653
6	82.034	81.8583	1.6941	79.6958	3.0653
7	50.278	50.0583	1.6941	45.5708	3.0653
8	91.056	90.8583	1.6941	91.8958	3.0653
9	65.345	65.1583	1.6941	72.8208	3.0653
10	63.334	63.4417	1.6941	64.8792	3.0653
11	60.301	59.1667	1.2671	59.1667	3.8897
12	94.796	94.8417	1.6941	99.0042	3.0653
13	57.017	57.1417	1.6941	44.1792	3.0653
14	88.70	88.8417	1.6941	90.5042	3.0653
15	57.300	57.4417	1.6941	52.6792	3.0653
16	21.755	21.8417	1.6941	19.4561	3.0653
17	31.739	31.8417	1.6941	30.2542	3.0653
18	61.127	61.2417	1.6941	63.2542	3.0653

SE = standard error

The full regression model relating the dissolution extent of PGN after 20 min to the SC-CO_2 processing conditions was generated from the factorial study and is shown in Eq. 3:

$$Y_2 = 67.030 + 2.232A + 3.360B + 6.889C + 1.306D + 2.853E - 0.949F - 0.633G - 1.382AB + 0.509AC - 0.007AD + 0.253AE - 5.865AF + 1.286AG - 5.660BD - 3.475 \quad (3)$$

The main effects showed that a high pressure (A) of 186 bar, high temperature (B) of 60°C, large sample load (C) of 9 g and more SC-CO_2 processing time (D) of 30 min all have positive effects on PGN dissolution over the first 20 min. Furthermore, using a higher pressure (186 bar) and a smaller orifice diameter (1/16″) also had an apparent effect on PGN dissolution over 20 min. The synergetic effect of these two variables was reflected in the interaction plot. Interestingly, the amount of PGN dissolution from the dispersion systems formed from a lower pressure (90 bar) and temperature (20°C) was much lower than from dispersion systems formed from the opposite conditions. High PGN dissolution over 20 min was also expected when the pressure was low in combination with a higher excipient amount. Finally, when a longer

processing time is used, a higher temperature must also be used in order to increase the PGN dissolution after just 20 min.

Y_3—Time to Dissolve 50% PGN ($t_{1/2}$)

In the factorial design, the time when 50% of the SC-CO_2 processed PGN dispersion systems had been dissolved ranged between 2.8 and 17.7 min, as shown in Table VII. Of the seven factors tested, four of the SCF processing variables had a significant effect on the $t_{1/2}$. Table VII shows that pressure (A), temperature (B), processing time (D) and orifice diameter (G) all had effects on PGN dissolution at the 50% time point.

Once again, both pressure (A) and temperature (B) were significant factors in the time it took to reach 50% PGN dissolution (response Y_3), just as they were for responses Y_1 and Y_2. The other factors that influenced the time to reach $t_{1/2}$ included the processing time (D) and the orifice diameter (G). The amount of sample loading (C), sonication (E) and PGN/excipient ratio (F) did not significantly affect the time it took to reach $t_{1/2}$.

The full regression equation derived from the factorial study relating to $t_{1/2}$ for PGN dispersion systems is shown in Eq. 4:

$$Y_3 = 8.041 + 2.778A + 1.125B + 0.223C - 1.878D + 0.321E + 0.033F + 0.966G + 699AB - 0.386AC - 1.765AD + 0.338AE - 0.245AF + 0.567AG - 0.709BS - 3.321 \quad (4)$$

The main effects showed that SC-CO_2 processing with a high pressure (A) of 186 bar, high temperature (B) of 60°C, short processing time (D) of 10 min and large orifice diameter (1/4″) all have positive effects on reducing time to reach 50% PGN dissolution. This partly is consistent with the previous sets of responses, where both pressure and temperature were also high, but the short processing time is the opposite to that found relating to PGN dissolution after 20 min. For the orifice diameter (G), this is the first time it was significant enough to be seen in the main effect to a response (Y_3), that is the orifice diameter was an insignificant influence on Y_1 and Y_2.

The interactions showed that four major interactions occurred. It was found that using a higher temperature (60°C) and more processing time (30 min) had evident effect on the time taken to reach 50% PGN dissolution. It was also clear that the higher the pressure in combination with higher temperature and larger orifice size was required to improve the $t_{1/2}$ period.

Table VII. Estimated Effects and Coefficients for $t_{1/2}$ for PGN Dispersions (R^2=100%)

Term	Effect	Coefficient	SE coefficient	T value	p value
Constant	–	8.041	0.03076	261.45	0.002
A	5.557	2.778	0.03076	90.33	0.007*
B	2.249	1.125	0.03076	36.56	0.017*
C	0.447	0.223	0.03076	7.26	0.087
D	−3.757	−1.878	0.03076	−61.08	0.010*
E	0.643	0.321	0.03076	10.45	0.061
F	0.066	0.033	0.03076	1.08	0.476
G	1.932	0.966	0.03076	31.41	0.020*
AB	1.397	0.699	0.03076	22.72	0.028*
AC	−0.771	−0.386	0.03076	−12.53	0.051
AD	−3.531	−1.765	0.03076	−57.40	0.011*
AE	0.676	0.338	0.03076	10.99	0.058
AF	−0.491	−0.245	0.03076	−7.98	0.079
AG	1.134	0.567	0.03076	18.44	0.034*
BD	−1.418	−0.709	0.03076	−23.05	0.028*
Centre points	–	−3.321	0.09227	−35.99	0.018

A = pressure, B = temperature, C = sample load, D = contact time with CO_2, E = sonication, F = drug/excipient ratio, G = orifice diameter

*p value ≤0.05, statistically significant variables (ANOVA)

DISCUSSION

Effect of Pressure (A) and Temperature (B) of SC-CO_2

As has been noted previously, the solubility of a drug in a SCF depends on a critical balance between CO_2 fluid density and the drug vapour pressure; both are controlled by temperature and pressure of the solvent fluid (1,34). In all three main responses, the temperature and pressure were found to be significant main factors. Temperature did have an interaction effects for both the PGN dissolution extent after 20 min and dissolution time for 50% of PGN (p value <0.05), but had no significant interactions for the process yield (p value >0.05).

A temperature increase can cause an increase in solvating ability of SC-CO_2 due to increasing the solute vapour pressure, even while reducing the fluid density (1,35). For the *in vitro* dissolution responses measured, the relationships between temperature and pressure and the other factors were more complicated than for the process yield. From the results, it can be inferred that the pressure of the SCF plays an important role in the formation of PGN dispersion systems using PGSS; in fact, a high pressure could be considered the most important factor for all the responses (36). It is well-known that a temperature from 40°C to 60°C as little impact on PGN solubility in SC-CO_2, while as pressure increases (from 120 to 210 bar) over the same temperature range, the increase in PGN solubility in SC-CO_2 is almost sevenfold (28,37). This means that process yield and dissolution are likely to be affected as pressure increases. As pressure increases, the fluid density increases and this could have two effects: an increase in the solvating ability of the SC-CO_2, but lower interaction between the drug and excipient as a consequence of the lower diffusion at higher density (1,31,38). There are few studies that have considered the effects of density and diffusivity of a SCF and PGSS method and the relationship with yield and *in vitro* dissolution, and no studies have determined the effects of different SCF conditions on the formation of PGN-loaded Gelucire 44/14 dispersion systems. In one study, the SCF conditions were examined in the formation of carbamazepine particles using a GAS method using 290 bar at 33°C produced the highest yields (73%), while lower temperatures yielded little or nothing (39). Another study showed that nifedipine can be processed using a PGSS-CO_2, and depending on the SCF conditions, the nifedipine dissolution rate was significantly increased by approximately double after 15–60 min (40). In a similar study, felodipine was processed using a PGSS method; however, the resulting dissolution rates in pure water were not significantly increased (41). Although there are numerous studies

investigating SCF conditions associated with forming different or possibly new formulations with certain physicochemical properties, there still remains limited information on mixing ability of a wide range of SCF conditions. This study has helped to establish some ground work towards a set of guides that could be used as a platform for a range of drugs and excipients.

Effect of Sample Loading (*C*)

In order to achieve high process yields, it was considered necessary to examine the amount of sample loaded into the sample cylinder. The maximum pressure of the sample cylinder is approximately 186 bar, which could be reached with approximately 294.8 g of CO_2. PGN solubility below the SCF point of CO_2 is less than 0.18 mole fraction—this provided overcapacity where the highest amount of PGN was 4.5 g or only a mole fraction of 0.015, which is 12 times less than the solubility at the SCF highest pressure level (28,29). Given the density of CO_2 at its critical temperature and pressure is 0.496 g/cm^3 and the sample cylinder had a volume capacity of 300 cm^3, the maximum sample loading (excipient and PGN) weight of 9 g was chosen in this study. This gave a 0.064 ratio of solid material to CO_2 at the minimum pressure and temperature to form CO_2 into a SCF. There are no studies that have investigated the effect on SC-CO_2 density/diffusivity associated with PGN loading of Gelucire 44/14.

Ironically, in this study, only the dissolution response measuring extent after 20 min appeared to hold a significant value. This indicates that either the loading range considered was ideal or that the parameters used did not test the physical boundaries to detect influence. It is possible that varying ratios of the loaded sample (*F*) (*e.g.* excipient to PGN) and orifice diameter (*G*) may have directly affected this variable (see "Y_3—Time to Dissolve 50% PGN ($t_{1/2}$)").

Effect of SC-CO_2 Processing time (*D*) and Sonication (*E*)

An advantage in the use of CO_2 in this study was its low polarity, making some degree of solubility of both PGN and excipient possible. In order to produce sufficient dissolution *via* improved dispersion, the process was conducted under static conditions (*i.e.* closed system); this allowed for a better penetration of the fluid in the excipient matrix than in a dynamic method where CO_2 is continuously added and released throughout the system. In this model experiment, the main effect of SC-CO_2 processing time (*D*) was found to be significant for all three main responses. The SC-CO_2 processing time (*D*) did not show significant interactions for the process yield, but there was a similar interaction between (*D*) and temperature (*B*) for both dissolution responses.

Effect of Excipient: Drug Ratio (*F*) and Orifice Diameter (*G*)

The excipient fraction of a formulation can be central in the dissolution of a drug; hence, varying ratios of excipient to PGN were investigated. For only the process yield the main effect of the variable (*F*) was significant with a positive coefficient, *viz* the increase in the excipient fraction to PGN lead to an increase in process yield. A single two-factor interaction with pressure (*A*) and (*F*) was also observed to be statistically significant. The interaction result shows a significant increase in yield when a high SCF pressure (186) was used in combination with a high drug/excipient ratio (1:10) (p value = 0.044). In hindsight, it was reasonable to expect that a smaller amount of drug would impact positively on yield, as larger amounts of drug can reduce the rate of SCF expansion and larger amounts of drug re-crystallization on expansion of the SCF would further impede yield. In one study, a combination of excipients such as PEG 8000, Gelucire 44/14 and vitamin E (TPGS) was tested using various ratios and found that tertiary mixtures such as one part PEG, four parts Gelucire 44/14 and one part TPGS performed better than binary systems (2). In this study, only binary systems in different ratios to drug were investigated, thus more research needs to be conducted to further understand the effects of different amounts and combinations of different excipients.

A possible drawback in the use of tubing between the sample cylinder and precipitation chamber is the absence of spraying ability. Nevertheless, this limitation may have been partly overcome by investigating different tubing diameters, such as 1/4 and 1/16, in order to increase or decrease the release size, angle and rate, hence mimicking spraying. Numerous studies have found that for particle size reduction, the nozzle assembly is a critical aspect in the design of a SCF unit (10,42,43). However, there are no studies investigating the effects of a nozzle on the formation of PGN-loaded Gelucire 44/14 dispersion systems. The model experiment, in this study found that only (*G*) had a significant main effect on $t_{1/2}$ and two possible interactions with pressure (*A*) for both the extent dissolution after 20 min and $t_{1/2}$. This indicates that the process yield was not affected by the orifice diameter and that dissolution may be improved further with use of more sophisticated nozzles that have enhanced spraying capabilities.

Another general limitation of this study was the inability to draw more accurate levels for each variable studied. That is the levels tested were only parameters used in the DOE such as a high pressure (186 bar), rather than identifying whether or not slightly lower or higher pressures could have produced more ideal responses. An optimization within and outside the model space of the DOE can be conducted using central composite designs and other quadratic (non-linear) models. No studies to date have investigated the formation of dispersion systems using SCF and quadratic experimental designs.

CONCLUSIONS

The formation of PGN-loaded Gelucire 44/14 dispersion systems using the PGSS method and SC-CO_2 was possible. In this investigation of the effects of the seven test parameters in the formation of PGN-loaded Gelucire 44/14 dispersion systems were evaluated using an experimental design approach. The optimal experimental conditions for the developed and constructed SCF unit employing the PGSS method were also found inside the experimental domain. The results show the significance not only of the main effects of pressure and temperature in the mixing of Gelucire 44/14 with PGN, but also of the interaction effects that would have been lost in the use of a conventional one variable at a time approach. It was found that all seven factors were significant for one or more of the responses investigated, but not all seven factors mattered for each response. High pressure (186 bar), temperature (60°C)

and processing time (30 min) all had positive effects on yield, E_{20} dissolution and $t_{1/2}$, except for $t_{1/2}$ where a shorter processing time of 10 min was more ideal. The higher loading amount of 9 g and longer sonication time of 10 min were only significant E_{20} dissolution, while the larger orifice size during expansion only affected the $t_{1/2}$. The lower drug-to-excipient ratio of 1 to 10 had a statistically significant influence on only the processing yield. It may be concluded that a higher pressure and temperature, larger sample loading, longer processing time, longer sonication duration, a lower drug-to-excipient ratio and larger orifice size during expansion are the optimal conditions for the preparation of PGN-loaded Gelucire 44/14 dispersion systems. Gelucire 44/14 dispersion systems processed using SC-CO_2 may be considered as a promising carrier for transdermal delivery of PGN.

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