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Original Research

Formulation Of Topical Self-Nanoemulsifying Drug Delivery Systems (SNEDDS) Of Vitamin D3

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ABSTRACT

Background: *In the last few decades, there has been a significant increase in customers' interest in skin care, including anti-aging. One of the skin care substances, vitamin D3, has a positive impact on the skin, such as the keratinocyte differentiation effect to maintain the skin barrier and the hydration effect to keep the skin moist. Vitamin D3 has a high lipophilicity, so it is considered ideal to formulate in the self-nano emulsifying drug delivery system (SNEDDS). The higher solubility of vitamin D3 in the SNEDDS oil component could improve its penetration through the skin. The SNEDDS is a primary dosage form that can be entrapped in semisolid base dosage forms, such as cream, lotion, or gel. SNEDDS vitamin D3 needs to be optimized to obtain the appropriate composition of the components: oil, surfactant, and co-surfactant.*

Methods: *The D-Optimal Mixture method using Design Expert 10 software had been chosen as an optimization tool for SNEDDS vitamin D3.*

Results: *The composition of the optimum formula was obtained as follows: 1.0351 g Miglyol 812 N; 3.0637 g Tween 80 and 0.9011 g PEG 400. The optimum formula has a particle size of 32.62 nm ± 1.80 nm and a polydispersity index of 0.31 ± 0.03.*

Conclusion: *The release test was carried out with the help of a Franz diffusion cell instrument, a cellophane membrane, and phosphate-buffered saline pH 7.4 containing 0.5% Tween 80. The cumulative amount of vitamin D3 released per minute (Flux) was 0.10 µg/min.*

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INTRODUCTION

There is an increasing trend in facial skin care, including anti-aging treatments (Ramos-e-Silva et al., 2013). Vitamin D is involved in the molecular pathway of the anti-aging process. It starts with 7-dehydrocholesterol in the keratinocytes, which is converted into vitamin D by ultraviolet (UV) light. The process is continued by the

enzymes CYP27A1, 25 hydroxylases, and CYP27B1, 1-hydroxylase, to convert vitamin D into its active metabolite, 1,25 dihydroxy vitamin D ($1,25(OH)_2D$).

The metabolites bind to the vitamin D receptor (VDR) and increase calcium to initiate keratinocyte differentiation and inhibit proliferation (Bikle, 2012) (Mostafa & Hegazy, 2015). Vitamin D also increases the cornified envelope and the expression of involucrin, transglutaminase, loricrin, and filaggrin (Mostafa & Hegazy, 2015). The cornified envelope is an element in keratin production. Loricrin, involucrin, and filaggrin are involved in the accumulation of keratohyalin granules for cell structure.

Those elements are pivotal in composing keratinocytes to form the epidermal layer as skin protection from environmental factors (Piotrowska et al., 2016). Vitamin D has an antioxidant and inhibitory effect on UV-induced cell damage (Philips et al., 2022). At the molecular level, vitamin D increases collagen and inhibits collagenases to maintain skin integrity (Dobak et al., 1994) (Philips et al., 2022).

Because the chosen substance, vitamin D3, is lipophilic, it should be formulated in a lipid-based drug delivery system (LBDDS). The nano-emulsifying drug delivery system (SNEDDS) is one of the LBDDS, consisting of oil, surfactant, and co-surfactant or co-solvent mixed in light agitation. When diluted by water, it forms refined droplets of oil in water called nanoemulsions (Mohsin et al., 2012).

The droplets contain active substances dissolved in the oil phase and increase the interfacial area of dispersion. As a result, the solubilization and permeation of a drug increase through the mechanism of transport characteristics. SNEDDS have a higher drug loading capacity than oil solutions due to the high concentration of surfactant and co-surfactant (Rehman et al., 2017).

To our knowledge, there are no SNEDDS of vitamin D3 for topical purposes. This research aimed to optimize the SNEDDS of vitamin D3 using these three components: miglyol 812 N as an oil phase, tween 80 as a surfactant, and polyethylene glycol (PEG 400) as a co-surfactant. The optimum formula was expected to fulfill the particle size specification to reach the target of the anti-aging treatment, the dermis layer.

The oil phase or a mixture of surfactant and co-surfactant affects the resulting particle size (Astuti, 2018). The selection of oil was based on its non-comedogenic properties, ability to soften the skin, occlusivity, and potential moisturizing effect (Lin et al., 2018).

MATERIALS AND METHOD

Materials

Vitamin D3 (cholecalciferol) (Xi'an Lyphar Biotech Co., Ltd., China), miglyol 810 (IOI Oleochemical, Germany), tween 80 (Kao Chemicals Global, Japan), PEG 400 (Kao Chemicals Global, Japan), and phosphate buffer saline (PBS) pH 7.4 (Corning, USA).

Instruments

Particle Size Analyzer (PSA) (Malvern, Instruments, Malvern, UK), High-Performance Liquid Chromatography (HPLC) (Hitachi LaChrom Elite D2000 L-Series, Japan), Franz Diffusion Cell, hotplate, and magnetic stirrer.

Methods

Pseudo-Ternary Diagram Phase

The pseudo-ternary diagram phase was constructed to obtain each SNEDDS component range. The diagram is used to obtain the equilibrium of three phases. In this case, the phases are oil, water, and emulator referred to as a mixture of surfactant or co-surfactant (Smix).

The ratio of Smix was chosen as 3:1, and then the Smix was mixed with oil in several ratios as follows: 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, and 1:9. The water was slowly added to the mixture while being moderately stirred with a magnetic stirrer. The volume of water was noted when the mixture reached a certain point: either it was turbid or clear. Based on this experiment, a range of SNEDDS components was obtained (Cui et al., 2009).

Optimization of SNEDDS of vitamin D3 using D-Optimal Mixture Design

The optimization was observed using D-Optimal Mixture in Design Expert 10 software. The results from the pseudo-ternary diagram phase were used as a starting range for each component, except the value of the water portion was transferred to the Smix value because SNEEDS is an anhydrous nanoemulsion. Three factors were processed in the D-Optimal Mixture as factors or independent variables: miglyol 812 N, tween 80, and PEG 400. The D-Optimal Mixture produced 16 runs of the three factors to be evaluated: response, particle size, and polydispersity index.

Formulation of self-nano emulsifying drug delivery system (SNEDDS) of vitamin D3

According to the suggested SNEDDS composition from the D-Optimal Mixture method, vitamin D3 was dissolved in the oil phase before adding the surfactant and co-surfactant. They were mixed with moderate agitation until a clear, transparent mixture was obtained (Cui et al., 2009).

Measurement of particle size and polydispersity index

Particle size and polydispersity index were measured simultaneously using a PSA at a temperature of 25 °C. The polydispersity index measurement aims to depict the distribution of particle sizes (Clares et al., 2014).

Release Test

The release of vitamin D3 from SNEDDS was evaluated using Franz Diffusion Cell at 37°C ± 2°C. The diffusion media was PBS, pH 7.4, containing Tween 80 at 0.5%. The cellophane membrane was immersed in water overnight to make it saturated. Vitamin D3 SNEDDS were weighed at 2 g and placed on the cellophane membrane.

The sample was pipetted at 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, and 300 minutes. Following the withdrawal of a sample, a volume of diffusion media was added to maintain the sink condition. The determination of vitamin D3 was measured by HPLC (Abd-Allah et al., 2010).

RESULTS

The pseudo-ternary phase diagram showed the area of each range of SNEDDS components (Figure 1). The mixture of Miglyol 812 N, Tween 80, and PEG 400 generates a small area, which means it has a narrow option for each component to form

a nanoemulsion. Based on the area, it can be deduced that the range for each component is 0.5386–1.874 g Miglyol 812 N, 2.4829–3.645 g Tween 80, and 0.5738–0.928 g PEG 400.

That range became the starting point for the optimization stage using the D-Optimal Mixture method in Design Expert 10 software. The range of values of SNEDDS' components as input to the software was automatically arranged into the 16 runs of combinations that needed to be evaluated (Table 1).

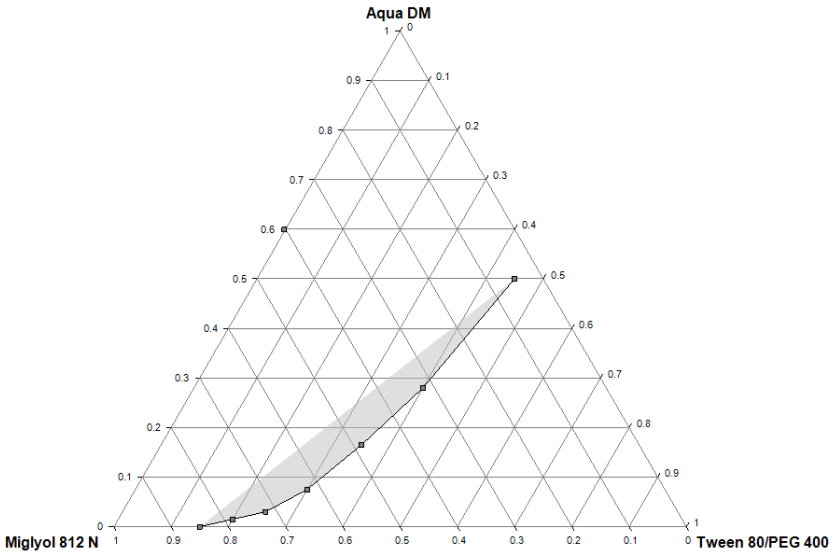

Figure 1. Pseudoternary Phase Diagram

Table 1. Experimental data and response using D-Optimal Mixture method

Run	Miglyol 812 N (g)	Tween 80 (g)	PEG 400 (g)	Particle Size (nm)	Polydispersity Index
1	1.09837	3.30023	0.601401	17.52	0.085
2	1.23505	3.03771	0.727248	22.95	0.160
3	1.46785	2.95835	0.5738	156.9	0.533
4	1.23505	3.03771	0.727248	23.68	0.191
5	0.538673	3.645	0.816327	19.29	0.236
6	0.892079	3.17992	0.928	15.13	0.142
7	1.66262	2.4829	0.854485	196.5	0.518
8	1.64501	2.72691	0.628074	222.8	0.466
9	1.87419	2.55201	0.5738	136.5	0.283
10	1.66262	2.4829	0.854485	179.8	0.451
11	1.23505	3.03771	0.727248	22.37	0.161
12	0.538673	3.645	0.816327	14.98	0.286
13	1.35614	2.71586	0.928	135.8	0.277
14	1.23505	3.03771	0.727248	25.38	0.208
15	0.880093	3.54611	0.5738	15.38	0.071
16	0.674505	3.39749	0.928	14.64	0.135

Furthermore, the data of the factors and their responses from Table 1 were analyzed to yield the final equations describing the effect of each factor on the response:

$$\text{Particle size} = 146.93A - 267.21B - 5770C + 726.12AB + 7586.36AC + 9029.07BC - 14838.93\,A^2BC - 26527.53\,AB^2C + 49876.99\,ABC^2 \quad \text{.....(1)}$$

$$\text{Polydispersity index} = 0.23A - 0.84B - 27.08C + 2.79AB + 35.39AC + 40.21BC - 57.69\,A^2BC - 79.55\,AB^2C + 175.53\,ABC^2 \quad \text{.....(2)}$$

Notes:
A = Miglyol 812 N
B = Tween 80
C = PEG 400

Those equations were used to build the model representing the response of particle size and polydispersity index (Figure 2). The following optimization step was used in predicting the optimum formula, which was conducted automatically by Design Expert 10 software. The acceptance criteria were set at 20–200 nm for the particle size and 0.2–0.8 for the polydispersity index. Several predictions of the optimum formulas were suggested, and only one was chosen to be continued to the verification and validation step (Figure 3). Another requirement was that the desirability of the proposed formula be 1.

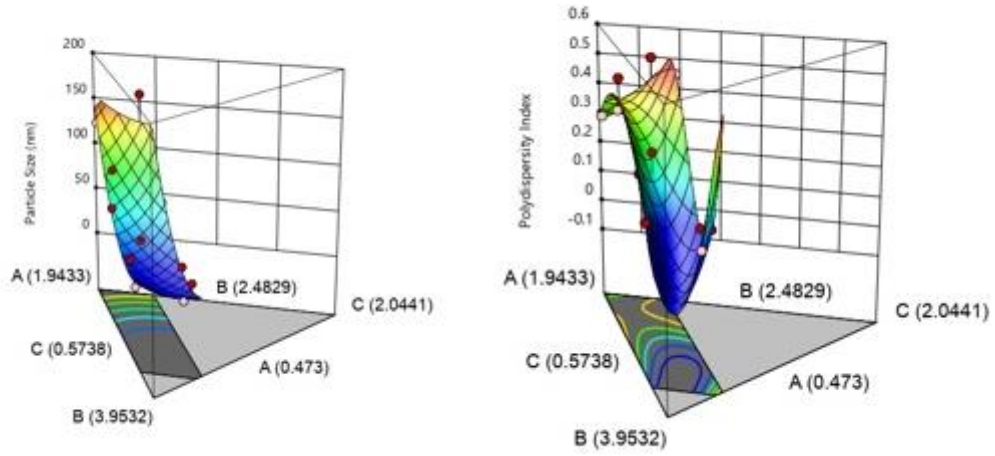

Figure 2. 3D diagram of the relationship between the variables of Miglyol 812 N (A), Tween 80 (B), and PEG 400 (C) to the responses: particle size (left), and polydispersity index (right)

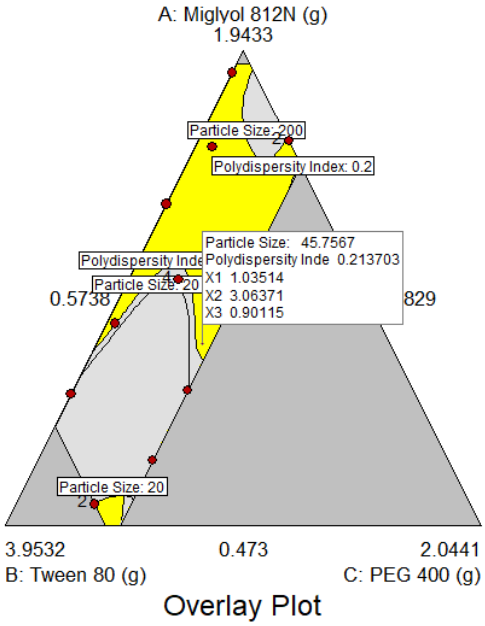

Figure 3. The composition of the predicted optimum formula

The predicted optimum formula needed to be verified and validated to ensure the actual values were within the acceptance range of the predicted values. The suggested optimum formulas were made again in triplicate and tested for their particle size and polydispersity index. The results were within the range of the 95% confidence interval (CI). The formula has been stated as valid for use as the optimum formula. As a result, the average values of the particle size and the PDI were 32.62 nm ± 1.80 nm and 0.31 ± 0.03, respectively.

Table 2. The predicted and actual values of the optimum formula

Predicted value		**Actual value**			**95% CI low for Mean**	**95% CI high for Mean**
		1	**2**	**3**		
Particle size (nm)	45.7843	30.56	33.39	33.90	30.3594	121.928
Polydispersity index (PDI)	0.213754	0.301	0.286	0.342	0.06179	0.365714

The release test of SNEDDS of vitamin D3 was done in triplicate (Figure 4). Samples pipetted from the Franz diffusion cell were analyzed using HPLC and read in units µg/mL. The release test results were calculated to obtain a cumulative amount of drug released per unit of time per unit of area (Q).

The correction factors of Wurster were applied to calculate the Q value (Handayani et al., 2012). Further, a correlation plot between Q vs. √t was constructed. The slope of the plot showed the flux value, or amount of drug released per unit of time, and it was stated as 0.1039 µg per min (Figure 4).

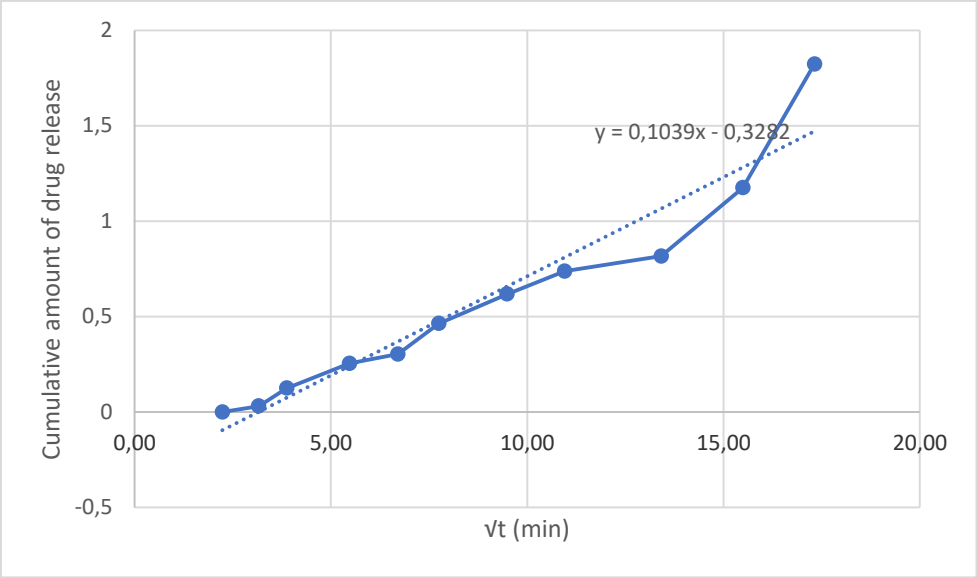

Figure 4. Release test profile of SNEDDS of vitamin D3

DISCUSSION

Figure 2 from the particle size test shows that a high Miglyol 812 N percentage gave the most increased particle size because of the oil phase forming the SNEDDS droplet and the nanoemulsion's internal phase. The 3D diagram also showed that the PEG 400 percentage did not become the particle size variable. Meanwhile, the largest particle size was yielded when the Tween 80 was below 3.0 g and vice versa.

The result was in line with the study by Guttoff et al., (2015) that found the high Tween 80 percentage produced a smaller particle size. If the surfactant concentration is too high, it will form a liquid crystal surfactant-oil-water (SOW) mixture that is hard to disperse and will produce bigger droplets. On the contrary, the SOW mixture will not be formed at a low surfactant concentration, generating a big droplet (Guttoff et al., 2015).

Miglyol 812 N and Tween 80 have their own specific range of percentages, resulting in the lowest polydispersity index (PDI). This finding is similar to the results of Guttoff et al., (2015) who found PDI was at the weakest points in the medium range of surfactants, even though the meaning of "medium range" is relative based on each characteristic of substances. Knowledge and control of particle size are essential factors in pharmaceutical dosage forms. Particle size, along with area, impacts the physicochemical characteristics, release profile, and pharmacological effect of a drug.

In this research, controlling particles helped increase the solubility of the drug, improving the amount of drug release from the dosage forms and delivering the drug to the target dermis layer. The release test was observed using the Franz Diffusion Cell, which is the main principle of a diffusion process. It is designed to have a donor compartment and an acceptor compartment; both are separated by the membrane, which serves as a limiting point of diffusion. There has to be a concentration gradient between those two compartments.

SNEDDS of vitamin D3 were put on the cellophane membrane as the donor compartment, while on the other side of the membrane, there was an acceptor compartment consisting of PBS pH 7.4 and Tween 80 0.5% (Xu et al., 2012). Vitamin D3 was dissolved in the SNEDDS droplet. The transport process of vitamin D3 can be described as follows: vitamin D3 was diffused across a SNEDDS droplet, then it had a partition across the cellophane membrane to the media PBS pH 7.4 and Tween 80 0.5%.

The significant component of PBS was water, where vitamin D3 has very low solubility. The addition of Tween 80 aims to increase the solubility of vitamin D3 in water. Any surfactant can be used as a solubilizing agent, such as trimethyl ammonium bromide (CTAB), sodium lauryl sulfate (SLS), and Tween. SLS has a more excellent drug dissolution enhancement than Tween (Fotaki et al., 2013). In this research, Tween was chosen due to the lack of bubbles produced by SLS.

CONCLUSIONS

The optimum formula of SNEDDS of vitamin D3 consists of 1.0351 g Miglyol 812 N, 3.0637 g Tween 80, and 0.9011 g PEG 400. It had a particle size of 32.62 nm ± 1.80 nm and a polydispersity index of 0.31 ± 0.03. Release testing using a cellophane membrane and PBS pH 7.4 (containing Tween 80 0.5%) as the media resulted in a flux value of 0.1039 µg/min.

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