Original Article

Effect of formulation variables on the properties of a new vesicular system of an anthraquinone derivative

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ABSTRACT

Drug delivery through the human skin is limited by the barrier function of the stratum corner. Several attempts have been investigated to enhance the transdermal permeation including vesicular structure. Diacerein is an anthraquinone derivative approved for osteoarthritis treatment. Owning to its oral side effects, transdermal delivery seems as an attractive approach. Vesicular carrier showed a promising results in enhancement of transdermal permeation. The present investigation aimed to screen the best surfactant and the optimum cholesterol-to-surfactant ratio needed for successfully entrapping the lipophilic investigated drug diacerein with the novasome. Diacerein novasomes has been successfully prepared by the thin film hydration method using different surfactant types. Also, Different cholesterol to span 60 ratio investigated. The vesicle size, PDI and EE% were determined for all of the prepared formulas. Results showed that span 60 as a surfactant gives the best results regarding entrapment efficiency owing to its unique physicochemical properties. A cholesterol to surfactant ratio of 1:4 gives superior entrapment efficiency. However, F1 needs further optimization by a suitable size reduction technique to produce a suitable a homogenous size distribution and an appropriate size range that is acceptable for transdermal delivery.

Keywords: Diacerein, Dermal delivery, Novasome, Surfactants

Introduction

The human skin is considered as the largest organ in the human body [1]. It exerts a protective function represented by the presence of the stratum corneum [2]. Therefore, unless the barrier property of the skin is manipulated, the low diffusion of the drugs across the stratum corneum is the major obstacle to topical delivery [3, 4].

Extensive research has been introduced to overcome stratum corneum and enhance drug permeation through the skin [5]. Vesicular systems are considered a wide area of investigation in these researches. Vesicle adhesion to the skin surface changes the structure of the stratum corner by fluidization of the lipid matrix is expected to result in this enhancement [6, 7]. Also, intact vesicular penetration related to their nano-size range allows close contact of the carrier with the skin [8, 9].

In the design of the vesicular system to enhance dermal permeation, it is essential to identify the skin layer intended as a target for drug deposition or whether crossing all layers of the skin is required so that drug molecules need to be in the viable epidermis to produce a beneficial effect as the case in transdermal delivery [10].

Conventional Vesicular systems such as liposomes and niosomes have several disadvantages in terms of drug loading and long-term stability [11]. Furthermore, these systems showed limited ability in crossing the skin layers and mainly deposited in the skin [12].

Novasomes (NS) are a new development of vesicular structure [13]. Their structure consists of non-ionic surfactants, cholesterol, and free fatty acids [14]. Surfactants are the basic unit and the building block of the vesicular structure that determines their structure and properties [6]. Cholesterol contributes to vesicular stability by increasing the transition temperature and

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altering the fluidity of the bilayer membrane [15]. Free fatty acids exert a permeation enhancement effect and increase the flexibility of the vesicular structure [16].

Novasomes (NS) have considerable exploitations in the arenas of cosmetics, personal care, nutrition, chemicals, agrochemicals, pharmaceuticals, and also, and they have been employed as adjuvants for human vaccines [17]. Recently, NS has been investigated as a valuable platform for the topical delivery of terconazole [18], Fluconazole [19], and fenticonazole [20]. NS was also investigated in the enhancement of transdermal permeation of agomelatine [21], nasal delivery of zolmitriptan [22], pulmonary targeting of terbutaline [23], and corneal penetration of fenticonazole nitrate [24].

The scope of the current research was to explore the best surfactant type and the optimum cholesterol level required to fabricate novasomes to act as a homing carrier for DCN dermal delivery.

Materials and Methods

Materials

Diacerein , brij 52 and brij 72 were purchased from Hangzhou Hyper Chemicals limited , China. Cholesterol (CH), span 60, span 40 and span80 were from Xi'an Sonwu biotech Co., Ltd, China, Stearic acid and Phosphate buffer saline pH 7.4 (PBS) were purchased from Himedia, India. Methanol, chloroform were obtained from alpha chemicals.

Methods

Preparation of DCN novasomes

DCN NS were prepared by the thin film hydration method. Formulas were prepared according to the composition shown in T**able 1**.

Weighted amounts of DCN, surfactants, cholesterol, and stearic acid were dissolved in a solvent mixture consisting of 10 ml chloroform and 5 ml of methanol. The mixture was sonicated for 10 min in a bath solicitor for complete dissolution. The resultant clear solution was added into a 250 ml round bottle flask that is attached then to a rotary evaporator. The solvents were evaporated by rotation at 100 rpm in a water bath warmed at 60 °C for 30 minutes. A vacuum was applied to aid in solvent evaporation till a thin clear transparent film was produced on the walls of the flask. Then, 20 ml of deionized distilled water was added and the flask is allowed to rotate at 150 rpm into a water bath previously wormed to 70 °C for one hour. The appearance of turbidity is an indication of novasomes formation. The dispersion was kept in the refrigerator at 4 °C overnight for complete annealing of the vesicular wall. Each formula was prepared in triplicate.

Table 1. The composition of DCN NS Formulation

F-code	SAA Type	Tc (°C)	HLB	Cholesterol: SAA ratio	Amount of cholestrol	Amount of SAA
F1	Span60	53	4.7	1:4	33.8	172
F2	Span60	53	4.7	2:4	67.6	172
F3	Span60	53	4.7	3:4	101.4	172
F4	Span40	42	6.7	3:4	101.4	161.04
F5	Span80	-12	4.3	3:4	101.4	171.44
F6	Bri 72	40	4.9	3:4	101.4	143.2
F7	Bri 52	36	5.3	3:4	101.4	132

*The amount of drug used in each formula was 10 mg, the volume of the hydration medium was 20 ml and the hydration time was fixed to 1 hour. Stearic acid was used as fatty acid at a fixed amount of 28.247 mg and SAA to FA ratio was kept constant at 1:0.25

Optimization of formulation variables

1. Effect of surfactant type

Five types of non-ionic surfactants with different HLB and physicochemical properties were used as a vesicle forming material. The surfactants used were span 60, span 40, span 80, brij 72 and brij52.

2. Effect of cholesterol to surfactant ratio

An appropriate balance between cholesterol and non-ionic surfactant is required to produce a stable vesicles with good entrapment efficiency . Three different ratio were investigated in this study represented by 1:4 , 2:4 and 3:4 cholesterol to surfactant .

Characterization of DCN novasomes

Determination of vesicle size and PDI

Dynamic light scattering (DLS) technology using Zetasizer Nano ZS (Malvern instruments, UK) was employed in order to determine the mean vesicle size of the prepared DCN novasomal dispersion. Appropriate dilution with distilled water (1:10) is required for obtaining suitable scattering intensity. Results were reported as mean ± SD [25, 26].

Determination of DCN entrapment efficiency (EE%)

The EE % of DCN with the prepared novasomal vesicles was estimated by ultrafiltration technique by measuring its free concentration within the supernatants [20]. The procedure

consist of taking 1mL of the dispersion into the upper chamber of a centrifuge tube matched with an ultrafilter (Millipore Company, USA, MWCO 10 kDa) and centrifigation for 30 minutes at 6000 rpm. Appropriate dilution of the ultrafiltrate with phosphate buffer is required to estimate the concentration of free unentrapped DCN by spectrophotometer at 258.8 nm. The EE% was calculated using equation [1]. Results were reported in triplicates as mean ± SD [27, 28].

Results and Discussion

The characterization parameters for the prepared DCN NS formulas were illustrated in **Table 2**.

Table 2. PS, PDI, and EE% of DCN-loaded Novasomes

F-code	Vesicle size (nm)	PDI	EE%
F1-Span 60(1:4)	2416± 162.025	1.101± 0.486	87.928 ± 0.348
F2-Span 60(2:4)	1032.33± 12.05	0.817±0.049	86.06±0.312
F3-Span 60(3:4)	855.96± 40.29	0.836±0.153	71.65±0.314
F4-Span 40	1325.66± 27.209	0.965±0.294	63.99±0.31
F5-Span 80	664.2± 15.818	0.568±0.0518	41.211±0.23
F6-Brij72	969.56± 13.56	0.685±0.35	67.64±0.3
F7-Brij52	1162.66± 54.123	0.72±0.45	58.55±0.034

Effect of formulation variables on EE%

1. Effect of surfactant type

To study the effect of surfactant type on the properties of the prepared novasomes, the cholesterol to surfactant ratio was adjusted at 3:4. This ratio was selected since Span 80 failed to produce vesicles at lower cholesterol levels [29]. Keeping all of the formulation variables constant, changing the surfactant type has a significant effect ($P < 0.05$) on the entrapment efficiency.

Span 60-based novasomes showed significantly higher EE% among all other surfactants investigated. On the contrary, Span 80 EE% was found to be the least. These results could be understood by examination of different physicochemical properties of surfactants used.

In the case of sorbitan monsters, the EE% was found to be in the order of span 60 (C_{18}) > span 40 (C_{16}) > span 80 (C_{18}). Both span 60 and span 40 are solid at room temperature owing to their high Tm, 53^0 C, and 42^0 C for span 60 and span 40, respectively. They share the same head group but differ in the alkyl chain length. However, the longer chain length of span 60 than span 40 (C_{16}) accounts for its higher lipophilicity and accommodation for lipophilic DCN resulting in higher EE% [30, 31]. On the other hand, the low transition temperature (T_c = 12 °C) and the unsaturated nature of span 80 resulted in increased vesicular permeability and drug leakage [32, 33].

In the case of polyoxyethylene alkyl ethers, the EE% of both brij 72 and brij 52 is significantly lower than span 60 (C_{18}). This order agrees with the different phase transition temperature of this surfactant (40 °C and 36 °C and for brij 72 and brij52, respectively as compared to 53°C of span 60). Research showed that as the phase transition temperature of the surfactant increase, the EE% will also increase [34].

2. Effect of cholesterol to surfactant ratio

Cholesterol accounts for vesicular membrane stabilization by enhancing its rigidity and decreasing drug leakages [35]. These effects result in the enhancement of the entrapment efficiency. Therefore it is necessary to optimize its level to obtain these effects. In order to study the effect of cholesterol inclusion with the DCN NS on its entrapment efficiency, three different Cholesterol: SAA ratios have been investigated in F1, F2, and F3. As the concentration of cholesterol increase from 1:4 to 3:4, the entrapment efficiency will be decreased. It is noticed that increasing cholesterol amounts behind certain limits may cause drug expulsion from the bilayer as they compete for packing space [36]. Also, disruption in the regularity of the It has been reported that beyond certain levels of cholesterol, the regularity of vesicular membranes occurs beyond a certain level of cholesterol a decrease in the EE [37].

Effect of formulation variables on vesicle size and PDI

1. Effect of surfactant type

The vesicle size was found to be in the following order span 40 >brij52 > brij 72> span 60> span 80. These findings could be explained based on the HLB of the different surfactants used. The highest HLB value of span 40 resulted in the largest particle size while the smallest HLB of span 80 resulted in the lowest particle size. It has been postulated that the vesicle size increase as the HLB of the used surfactant is increased. This is rationalized in terms of surface free energy that increases with a higher HLB value [29, 38].

2- Effect of cholesterol to surfactant ratio

In order to study the effect of cholesterol inclusion with the DCN NS on its particle size, three different cholesterol: surfactant ratios have been investigated in F1, F2, and F3. It is obvious that a reduction in particle size is associated with an increased cholesterol ratio from 1:4 to 3:4. The hydrophobic nature of cholesterol increases the hydrophobicity of the vesicular membrane causing a decrease in the surface free energy and smaller particle size will result [39]. This decrease in vesicle sizes statistically significant. Similar outcomes were also reported by other researchers [21].

Conclusion

According to the results obtained from the current study, sorbitan monostearate (span 60) was found to give the most promising results regarding entrapment efficiency of discern owing to its HLB and transition temperature. Also, the optimum cholesterol level was found to be 1:4 of surfactant concentration.

Recommendations

The selected formula (F1) needs to be subjected to a suitable size reduction technique to produce uniform PDI and a suitable size for transdermal application.

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