



Development of Solid lipid nanoparticles as Eschar delivery system for Nitrofurazone Using Taguchi Design Approach

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ABSTRACT

Effective topical antimicrobial agents decrease bacterial colonization and infection. Localization of nitrofurazone into the eschar using vehicle such as solid lipid nanoparticles (SLN) improves antimicrobial efficiency in treatment of the infection in burn patients. This study was undertaken to develop solid lipid nanoparticles (SLN) of nitrofurazone using cold homogenization technique and study the permeability of the drug through rat skin. Results show type and percentage of surfactant, type of lipid and drug/lipid ratio can influence SLN character Drug release from SLN was the rate limiting step for drug permeation across rat skin and so prepared formulations can act as a drug reservoir.

Keywords: SLN; Taguchi design; percutaneous absorption; entrapment efficiency.

Introduction

The burn eschar consists of denatured protein and avascular debris with fat necrosis, which provides an ideal environment for the growth of microorganisms (Monafa WW, West MA., 1990). In the absence of effective antimicrobial agents, gram's positive microorganisms proliferate and tissue eschar is suppressed by gram's negative species after 3 to 7 days (Bowler PG, Davis BJ., 1990). These infections are responsible for human mortality and morbidity and often result in prolonged hospital stay (Green JW, Wenzel RP., 1977). In the other hand eschar tissue is metabolically passive and contains heat-derived products, toxins and pathogenic micro-organisms, which may diffuse into the circulation, causing organ dysfunction and sepsis. Many of these problems can prevent by early excision of the eschar, a procedure that removes the agents responsible for immunosuppressant and sepsis (Williams WG, 2002).

Although early burn excision is an ideal solution for remove the immunosuppressive agent, but in some cases injuries to other organs prevent early operation (Ross DA et al., 1993).

Therefore, when early excision of the eschar is impossible, treatment of eschar infection with effective topi-

cal antimicrobial agents can help to decrease the bacterial colonization in the tissue, which would otherwise lead to decline the mortality and morbidity (Naoun JJ et al., 2004).

Because the site of action of antimicrobial products is not only the wound surface, therefore for prevention of bacterial and fungal invasions in burn tissues, the antimicrobial agents should be able to penetrate and localize in the eschar (Schwartz K., 2002). Nitrofurazone is one of the most frequently used topical agents in the treatment of burn wound infection with low molecular weight and high permeability through eschar (Stefanides MM et al., 1975).

Localization of nitrofurazone into eschar using vehicle such as solid lipid nanoparticles (SLN) improves antimicrobial efficiency in treatment of the infection in burn patients. SLN have been shown to have superior advantages over polymeric nanoparticles, fat emulsion and liposomes (Pardeike J et al., 2009; Schafer-Korting M et al., 2007). Moreover, the distribution of such drugs within SLN is effective due to their precutaneous uptake (Muller RH et al., 2000).

Drug release profile from SLN indicates two sections, burst and sustained released. Burst release might increase the percutaneous penetration hence, sustained release is important to supply the skin over a prolonged period of time (Pardeike J et al., 2009). Therefore, drug delivery with SLN provides fast and prolonged effects. This feature is beneficial in burn infection treatment. Burst release promotes antimicrobial effect of nitrofurazone in the early stage of burn and sustained release obtains prolonged effect and drug localization into eschar.

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This study was undertaken to develop solid lipid nanoparticles (SLN) of nitrofurazone using cold homogenization technique and study the permeability of the drug loaded in two types of SLN include SLN containing SLS (SLN1) and SLN containing Tween 80 (SLN2) through rat skin.

Material and methods

Materials

Nitrofurazone was purchased from Aldrich (USA), bees wax, cetyl alcohol, sodium lauryl sulfate, Tween 80 from Merck (Germany), Compritol® 888 ATO from Gattefossé (France), propylene glycol and polyethylene glycol 300 from Fluka Chemie AG (Switzerland), carbomer 934 from sigma St. Louis, MO, (USA) and dialysis bag from Toba Azema Co, Tehran (Iran). All chemicals were analytical grade or spectroscopic grade. Mini-tab14 software was used for experimental design and the evaluation of the effect of variables on responses.

Animal experiments

Male Wistar rats, weighing 260-340 g were used for in vitro permeation study. The experiments were performed in accordance with the guidelines for animal use of Ahvaz Jundishapur University of Medical Sciences. The guidelines were used prepared by the National Academy of sciences and published by the National Institutes of Health. After sacrificing the rats by ether, the abdominal skin removed with electric clipper and a razor without breaking the skin. Any extraneous subcutaneous fat was removed from the dermal surface. The full skin thickness was measured using digital micrometer.

In vitro permeation study

Home-made diffusion cells with effective area of approximately 2.49 cm² were used for permeation studies. Skin samples were hydrated before using in permeation study. Full skin samples were placed between donor and receptor chambers of the cells while the epidermal side faced the donor compartment. The donor phase was filled with 3ml saturated aqueous solution of drug as control and SLN formulations as test. Then receptor compartment was filled with buffer phosphate (pH 7). Temperature was maintained at 37°C ±0.5 and the receptor was stirred at 300 rpm. At pre-determined time intervals suitable amount of receptor solutions were withdrawn and immediately replaced

with an equal volume of fresh buffer. The cumulative amount of nitrofurazone passing across rat skin was calculated using the UV at 375 nm. The amount of drug passed across the rat skin and percentage were estimated after 32 h (Q₃₂). The temperature of diffusion cells did not affect skin permeability in the duration of experiments, which has been claimed in previous researches (Kalariya M et al., 2008; Panchagnula R et al., 2005).

Solubility determinations

The solubility of nitrofurazone on buffer and water was studied by equilibrating the suspension of excess amount of drug in 5 ml of medium. Then the solution shaken gently for 24 hrs at 32°C, centrifuged for 10 min at 3000 rpm, filtered, diluted and analyzed by UV.

Preparation of Nitrofurazone loaded SLN

Nitrofurazone loaded SLN were prepared by cold homogenization technique reported by Liedtke *et al.* Lipid compound (10% of total weight of dispersion) was melted. Then nitrofurazone and half of the total amount of surfactant dispersed and stirred in it and followed by sonication (90 W for 2 min). The fused lipid phase was scattered in aqueous solution contains co-surfactant and the rest of surfactant at 4°C (final volume was 50ml). Then the suspension is passed through homogenizer applying 3 cycling (20 s for each cycle) at 2000 bars. Taguchi orthogonal design with four independent variables include type of lipid, drug to lipid weight ratio (D/L), concentration of surfactant, and type of co-surfactant at three levels (Table 1) were used to achieve SLN with suitable properties same as encapsulation efficiency, release rate and particle size. In each formulation (F) one kind of lipid was used and the weight ratio of drug to lipid was applied as an independent variable.

Particle Size Determination

The mean particle diameter and polydispersity index (PDI) of SLN were calculated by laser light diffractometry, using Malvern Master Sizer SM 2000K (High Performance Particle Sizer; Malvern Instruments Ltd., Malvern, United Kingdom). Samples were prepared by dispersing nitrofurazone-loaded SLN with sufficient amount of water. The samples were stirred and followed by sonication for 2 min for resuspending SLN in medium.

Table 1: Independent variable and their levels in Taguchi experimental design

Independent variable	Levels		
	1	2	3
Type of lipid	Cetyl alcohol	Bees wax	Compritol
Drug: lipid weight ratio	1:4	1:8	1:12
Concentration of surfactant	8%	10%	12%
Type of cosurfactant	PG (10%)*	Carbomer (1%)	PEG* 300(5%)

* PG= propylene glycol, PEG= poly ethylene glycol

Scanning Electron Microscopy of SLN

Surface morphology and stability of SLN were studied by SEM (VP-1455, LEO, Germany). The scanning electron microphotographs were taken using a double adhesive tape applied on the aluminum dies and SLN were spread uniformly on it.

Encapsulation Efficiency

The percentage of encapsulated nitrofurazone (entrapment efficiency) was determined by spectrophotometric determination at 375 nm after centrifugation of the aqueous dispersion. The amount of free drug was detected in the supernatant. Then the amount of encapsulated drug was calculated as a result of the initial drug minus the free drug. The entrapment efficiency calculated by the following equation:

$$EE (\%) = \left(\frac{W_{\text{initial drug}} - W_{\text{free drug}}}{W_{\text{initial drug}}} \right) \times 100$$

" $W_{\text{initial drug}}$ " is the mass of initial drug used for the assay and the " $W_{\text{free drug}}$ " is the mass of free drug detected in the supernatant after centrifugation of (13).

Nitrofurazone Release Experiments through SLN

The nitrofurazone release studies were performed with static Franz diffusion cells (with 2.49 cm² surface area). Diffusion cells thermo regulated with water jacket and temperature was maintained at 32 °C ± 0.5. Then the receptor was stirred at 300 rpm. Buffer phosphate (pH 7) as receptor phase and 2 ml of SLN suspension as donor phase were used. Cellulose acetate membrane (0.1 μm pore size) was employed between donor and receptor phases. Samples were picked up in determined intervals over 24 h and analyzed by spectrophotometric at 375 nm (Saoto EB et al., 2004). Drug released after 24 h (R_{24}) have been reported and used as response in Taguchi experimental design.

Results and Discussion

Validity of nitrofurazone measurement method

Nitrofurazone has maximum light absorption in higher range of 300 nm which itself caused reduction the in-

terference light absorption. The relationship between the light absorption values are estimated by the concentration, was significant ($R^2 = 0.993$, $P < 0.001$). Repeated surveys accountability in measurement methods within and between days for nitrofurazone is represents the desired repeatability of measurement method on different days and caused nearly the same operation as well as error free results. The difference between real numbers and the estimated concentrations was about 5% that indicates the closeness of the estimated values to real results.

Determine water saturation concentration of nitrofurazone

The nitrofurazone saturation concentration in water and buffer phosphate (pH 7) was 19.7 ± 1.75 mg/ml (n = 3).

Encapsulation Efficiency

The percentage of the SLN loaded drug which prepared by SLS and Tween80 is presented in Table 2. For SLN1, the D/L and type of lipid were effective on the loaded amount of the drug. Among the used lipids, compritol, honey bees wax, and in the final stage cetyl alcohol were the most loaded. Also, the effect of lipid to drug ratio significantly affected the loading amount of drug, so the loading amount was high according to the increase in the lipid to drug ratio. It seems that the lipids of interest have good solubility capacity for nitrofurazone and did not limit the drug loading process.

For SLN2, the correlation between drug loading with D/L ($P = 0.0001$) and percentage of the surfactant ($P = 0.004$) was significant. This means that improvements in drug loading were observed with an increase in D / L ratio and percentage of Tween 80. In SLN2 formulations, the effect of type of the lipid on the loading amount was not significant. The analysis of the lack-of-fit represents that more than 99% percent of total data is estimable by D/L and percentage of surfactant.

To estimate and compare the effect of any surfactant on loading amount, the mean loading percentage of two surfactant were compared ($P = 0.05$) (Table 2).

Table 2: Nitrofurazone Loading efficiency in SLN1 and SLN2 (Mean ± SD, n = 5)

Formulation No.	Mean of Loading percentage		
	SLN1	SLN2	P value
1	17/82±0/65	23/08±0/95	0/002
2	27/76±1/2	1/85±43/2	0/000
3	47/5±1/35	2/14±54/1	0/011
4	24/72±0/88	1/05±21/44	0/014
5	62/4±2/51	1/45±45/76	0/000
6	51/3±1/87	0/57±39/5	0/000
7	33/5±1/2	0/66±19/4	0/001
8	56/1±2/30	0/85±31/52	0/000
9	64/7±3/90	1/47±52/55	0/001

Abbreviation; SLN containing SLS (SLN1), SLN containing Tween 80 (SLN2)

Based on the results, the difference of loading percentage between total drug in SLN1 and SLN2 was significant ($P < 0.000$).

In F4 to F9, loading percentage in SLN1 was more than SLN2, while in F1 to F3 was vice versa. The common factor in the F1 to F3 was the type of lipid and in case of all formulations was cetyl alcohol. In other words, when the cetyl alcohol was used, Tween 80 increased the drug loading amount in SLN.

But in other formulations in which the two other lipid, honey bees wax and compritol were used, in the presence of co-surfactants actually the loading amount in SLN1 was more than SLN2. In SLN1 the maximum loading amount was 64.7% (F 9), but in SLN2 was 54.1% (F 3).

Actually, the selection criterion for surfactant could be the type of the used lipid in formulations SLN1 and SLN2, that were compritol and cetyl alcohol, respectively. As Tween 80 seems to have more affinity to lipid phase, so it influence within cetyl alcohol is more than SLS and this increase the loading amount of the drug in cetyl alcohol.

Compritol is a polar lipid that tends to surfactant with higher HLB. Therefore, the SLS distribution in compritol is more than Tween 80. Thereby the drug loading amount has been more in case of SLS. It should be mentioned that in this study cold homogenization method is used for the preparation of SLN, herein the cold method comparing to hot method increased loading capacity of hydrophilic drug in SLN (Pardeike J et al., 2009).

Drug release from SLN

Nitrofurazone release from formulations of SLN1

The surfactant percentage ($P = 0.016$) and almost lipid type ($P = 0.05$) showed significant correlation with R_{24} . Thus the percentage of R_{24} reduced due to increasing in the amount of SLS. The lipid type also influenced R_{24} in such a way that compritol did, that had negative correlation with R_{24} and reduced R_{24} . The compritol, honey bees wax and cetyl alcohol increased the R_{24} . Cetyl alcohol that caused the lowest loading here creates the highest R_{24} .

Nitrofurazone release from formulations of SLN2

The D / L ratio and surfactant percentage showed significant correlation with the R_{24} ($P = 0 / 015$ and $P = 0 / 000$, respectively). Thus, increasing the D / L ratio and the percentage of surfactant reduced R_{24} . The R_{24} of the loaded drug for SLN1 & 2 are presented in Table 3. Profile of drug release from SLN1 was similar to SLN2 and includes a burst and slow stage in drug release mechanism (Fig 1 & 2). This profile may indicate the drug - enriched shell and core – shell model, in loading the drug. However, the loading model for both surfactants was the same and type of surfactant cause no displacement in the model. Drug release from SLN de-

pendents to drug properties, drug incorporation model, partition coefficient of drug between solid fat and water environment, molecular size, melting point of lipid and drug and the length of release pathway (Lukoneski G et al., 1997).

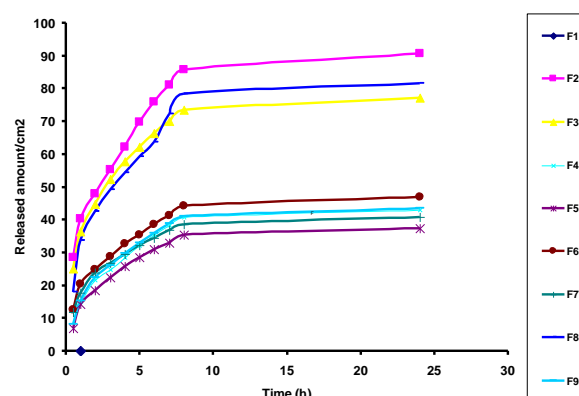


Figure 1: Nitrofurazone release profile from SLN1

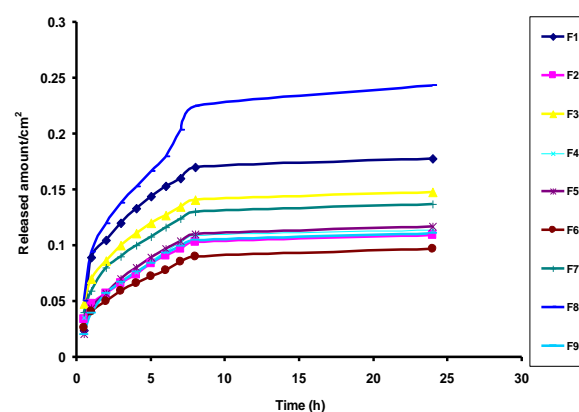


Figure 2: Nitrofurazone release profile from SLN2

The loading amount of prednisolone in the SLN containing cholesterol was 71% without all of burst, which within 5 weeks 83.8% of the drug was released. In the SLN prepared with compritol the loading percentage was 80%, while after 5 weeks only 37.2% of the drug is released without burst effect. The cholesterol and compritol have different melting point and in previously research has been declared that the lower melting point lipid can actually create a better slow release effect. This effect is due to the interaction between the drug, the surfactant and drug solubility associated with the melted lipid (Zur Muhlen A., Mehnert W., 1995). Because prednisolone has a higher melting point than lipid, therefore forms solid earlier than lipid and contains drug core and a lipid coating that prevents the rapid drug release (Zur Muhlen A., Mehnert W., 1995; Zur Muhlen A et al., 1998).

But in the present study because the melting point (238-242°C) of nitrofurazone is higher than three lipids, thus in the process of preparing SLN, in the lipid phase the drug is solid. Therefore, in such circumstance same as prednisolone, nitrofurazone also located in the core and a lipid phase forms around. Furthermore, a part of nitrofurazone is loaded in external part of the nanopar-

Table 3: Comparing the R₂₄ Mean between SLN1 and SLN2 (Mean ± SD, n = 3)

Formulation No.	R ₂₄		
	SLN1	SLN2	P. value
1	85.87 ± 6.27	80.11 ± 7.29	0.376
2	3.49 ± 78.19	5.11 ± 63.99	0.028
3	1.54 ± 45.41	2.95 ± 42.11	0.311
4	5.11 ± 67.85	4.86 ± 69.44	0.351
5	1.84 ± 37.73	1.67 ± 45.66	0.012
6	2.45 ± 65.03	3.54 ± 70.41	0.119
7	3.05 ± 33.44	3.94 ± 50.22	0.005
8	2.98 ± 60.24	5.41 ± 75.22	0.005
9	4.88 ± 57.69	2.59 ± 67.87	0.0410

Abbreviation; SLN containing SLS (SLN1), SLN containing Tween 80 (SLN2)

Table 4: Comparison of the particle sizes and polydispersity index (PDI) between SLN1 and SLN2

F No.	SLN1		SLN2		P-value
	Particle size(nm)	PDI	Particle size(nm)	PDI	
1	397 ± 25	0.250	426 ± 21	0.34	0.182
2	444 ± 33	0.320	475 ± 38	0.28	0.219
3	593 ± 18	0.21	571 ± 40	0.31	0.427
4	484 ± 45	0.30	505 ± 34	0.23	0.259
5	651 ± 28	0.34	668 ± 25	0.40	0.469
6	24 ± 501	0.19	481 ± 30	0.26	0.395
7	53 ± 629	0.29	611 ± 41	0.37	0.411
8	19 ± 477	0.35	490 ± 32	0.29	0.361
9	31 ± 465	0.37	455 ± 40	0.36	0.743

Abbreviation; SLN containing SLS (SLN1), SLN containing Tween 80 (SLN2)

icles that affects the burst release. Generally, the nitrofurazone model seems to be a homogenous model, but due to the low lipophilicity of the drug and fast distribution from lipid to water burst effect is seen. This hypothesis needs a support by thermomicroscopy evaluation.

The distribution of particle size of SLN formulations

SLN particle sizes of different formulations and polydispersity index (PDI) of SLN1 and SLN2 is presented in Table 4. Surfactant percentage had a significant correlation with particle sizes of SLN1 in a way that increase the percentage caused increase of the particle sizes (P = 0.034). The same result also has been observed in case of SLN2 (P = 0.039).

Although, some studies claimed that the use of short-chain fatty alcohol such as cetyl may reduce particle sizes (Zur Muhlen A., Mehnert W., 1995), but in the present study type of lipid has no significant effect on particle size. In the present study, increased concentrations of surfactant have been induced increase particle size, but in a study on the SLN formulations containing methotrexate different results have obtained (Kalariya M et al., 2008).

Evaluation of the nanoparticles surface morphology by SEM

SEM of SLN1 and SLN2 formulations are presented in figures 3 and 4, respectively. Both formulations showed smooth surface. It seems that the type of surfactant did not effect on particles morphology and dispersion in medium.

Equation 1: The illustration of the SEM of the lipid solid nano-particles of nitrofurazone containing SLS (1000 resolutions)

The effect of F7, F8 and F9 contain SLS on nitrofurazone permeability through rat skin in comparison with saturated aqueous solution of drug as control was evaluated. For this purpose percentage of drug and drug permeated across unit area after 32 hr (Q₃₂) was calculated (table 5). The Q₃₂ amount and percentage of passed drug in F7, F8 and F9 were significantly less than control which means All three Fs have been able to reduce the speed and amount of passed drug through the rat skin (P < 0.05). Among all, F7 showed the minimum Q₃₂ and percentage of the passed drug, while F8 had the highest. The highest surfactant has been used in the F7 and the F8 had the minimum used amount. Also the highest D / L ratio related to the F7 and the lowest was in F9. Highest and lowest R₂₄ values were observed in F8 and F7, respectively. The dif-

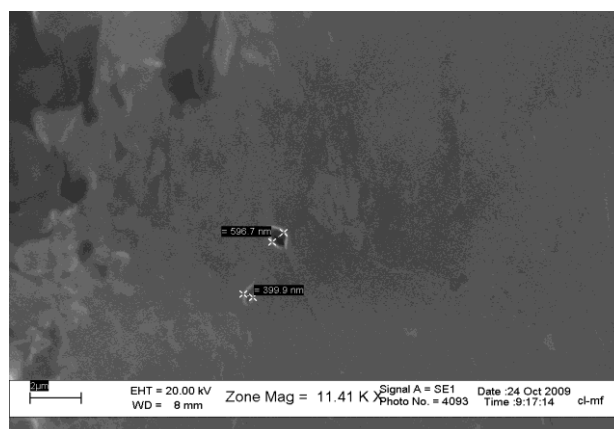


Figure 3: The illustration of the SEM of the lipid solid nano-particles of nitrofurazone containing SLS (1000 resolutions)

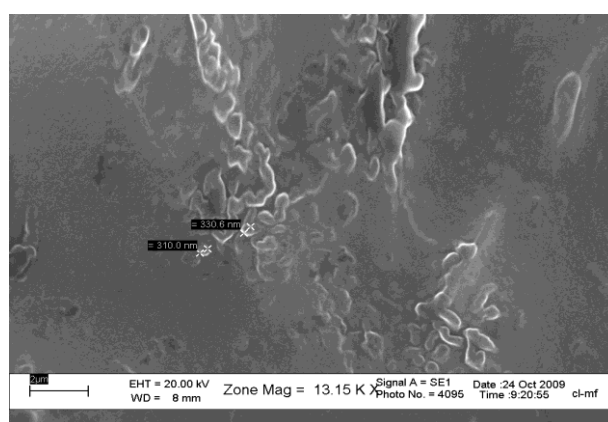


Figure 4: The illustration of the SEM of the lipid solid nano-particles of nitrofurazone containing Tween 80 (1000 resolutions)

Table 5: Cumulative amount of the passed drug through the skin unit (mg/cm²) from SLN formulations after Q32ours comparing to the control (Mean ± SD, n = 3)

F No.	Drug concentration in donor phase (mg/mL)	Q ₃₂	Percentage of the Passed drug after 32 hours
Control	220	18.44 ± 1.21	20.87 ± 1.33
7	0.280	0.003 ± 0.0002	2.66 ± 0.19
8	0.240	0.0073 ± 0.0003	7.58 ± 0.24
9	0.21	0.0002 ± 0.0051	5.99 ± 0.33

ference of Q₃₂ and the percentage of passed drug between three formulations was significant (P < 0.05). Thus, F8 which actually had the highest R₂₄ showed the highest Q₃₂ and percentage of passed drug. The total contents can be concluded that such rate limiting passed drugs through the skin is the stage releasing from the carrier, because the formulation that had more release also had highest Q₃₂ and greater release percentage. The R₂₄ release has negative and significant correlation with the surfactant percentage, so R₂₄ reduce due to increase in percentage of the surfactant.

Therefore, regulating the surfactant percentage may control the release rate and finally the skin permeability. On the other hand, since the R₂₄ in the preparation

of SLN2 in F7, F8 and F9 was more than SLN1, so to reduce R₂₄ and thus skin absorption rate is better to use less percentage of SLS in the SLN.

Conclusion

In conclusion, formulation parameters such as type and percentage of surfactant, type of lipid and D/L can influence SLN characters (entrapment efficiency, drug release amount and profile and particle size). Drug release from SLN was the rate limiting step for drug permeation across rat skin and so prepared formulation can act as drug reservoir in the skin especially in eschar with impair barrier properties.

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