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Research Article

In-vitro Permeation of Dexamethasone Microemulsion Through Rat Skin

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Abstract: Dexamethasone is a corticosteroid that prevents the release of substances in the body that causes inflammation. Dexamethasone is used to treat many different inflammatory conditions such as allergic disorders, breathing problems, ulcerative colitis, arthritis and skin conditions such as lupus erythematosus and psoriasis. The increased incidence of inflammatory diseases has necessitated the need to search for new topical dosage form of dexamethasone. This research was aimed to formulate, characterize and evaluate *in vitro* skin permeability of dexamethasone-loaded microemulsion systems as a topical delivery system of dexamethasone**.** Microemulsion formulations were prepared by mixing of appropriate amounts of surfactant including Tween 80 and Labrasol, cosurfactant such as Capryol 90 and oil phase including Labrafac lipophilicWL and Transcutol P (10:1). Physicochemical characterization of selected microemulsions including particle size, stability, viscosity, refractory index (RI), pH and in vitro skin permeability through rat skin using diffusion Franz cells were evaluated. The mean droplet size range of microemulsion formulation was in the range of 5.09 to 159 nm, and its refractory index (RI) and pH were 1.44 and 7, respectively. Viscosity range was 57-226 cps. Drug release profile showed that 48.18% of the drug released in 24 hours of experiment. Analysis of variance showed no significant differences between independent variables and J_{ss}, P, T_{lag} and D_{app} parameters of dexamethasone formulations. It can be concluded that a lower surfactant/cosurfactant ratio, improves the most permeability parameters of dexamethasone microemulsion.
Keywords: dexamethasone, microemulsion, permeability.

INTRODUCTION

The term microemulsion was originally proposed by Hoar and Schulman in the earliest of the 1940s. They generated a clear single-phase solution by titrating a milky emulsion with hexanol [1]. Microemulsions are homogeneous, transparent thermodynamically stable dispersions of water and oil stabilized by a surfactant, usually in combination with cosurfactant and whose diameter is in the range of 10-140 nm [2]. Conventional microemulsions are clasascan be categorized to oil-in-water (O/W), water-in-oil (W/O) and bicontinuous phase microemulsions [3]. The role of microemulsion is providing novel solutions to overcome the problems of poor aqueous solubility of highly lipophilic drug compounds and producing high, more consistent and reproducible bioavailability[4]. Human skin is an important target site for the application of drugs. Permeation of drugs through the skin is the basis of transdermal delivery. Transdermal drug delivery is associated with some advantages such as controlled drug delivery, continuous drug delivery, first-pass intestinal and hepatic bypass, avoidance of the gastrointestinal irritation, which is common with oral medications, and facilitation of drug localization at target site [5]. Drug permeation across different skin layers is affected by various factors such as physicochemical properties of the drug, vehicle and formulation components. Due to greater solubility of drugs and possibility of altering affinity of drugs to stratum corneum, microemulsions are able to increase transdermal absorption of lipophilic and hydrophilic drugs in topical drug delivery [6,7]. Dexamethasone is a synthetic, poorly soluble and crystalline corticosteroid that suppresses inflammation and normal immune response [7]. It is widely used as a therapeutic agent in alcohol withdrawal syndrome, cerebral edema, congenital adrenal hyperplasia, nausea and vomiting specially associated with high dose of anticancer agents, high altitude disorder, cerebral malaria, opportunistic mycobacterial infections, respiratory disorders, skin disorders rheumatism, meningitis, early mild carpal tunnel syndrome and as a diagnostic agent in Cushing syndrome [8]. Although, the correlation between microemulsion structure and composition and successful topical and transdermal drug delivery is not fully explained but a few studies have presented knowledge on interaction of the inner structure of the microemulsion and drug penetration into the skin [7,9]. The present study is an attempt to design various microemulsion formulations of dexamethasone for

topical and transdermal application. In the present study, the *in vitro* permeation of dexamethasone from microemulsion containing 0.1% dexamethasone have been investigated, and then compared with its aqueous saturated solution. The effect of surfactant/cosurfactant mixing ratios, oil phase and water on *in vitro* permeation was also evaluated using abdominal rat skin.

MATERIALS AND METHODS

Dexamethasone powder was bought from Darou Pakhsh Company (Iran). Tween 80 and PG were obtained from Merck (Germany), Caprylocaproyl macrogoglycerides (Labrasol), capryol 90, Median chain triglycerides (Labrafac Lipophile WL 1349) and Diethylene glycol monoethyle ether (Transcutol P) were kindly donated by Gattefosse Company (France). All chemicals and solvents were of analytical grade. All chemicals and solvents were of analytical grade. Freshly double distilled water was used in the formulations.

Assay

The quantitative determination of dexamethasone was performed by UV spectrophotometry at (BioWave II, WPA) λ_{max} = 244 nm.

Preparation of Microemulsions

The major determinant parameters in microemulsion preparation include surfactant/cosurfactant ratio (S/C), water content (%w) and oil percent (%oil). Full factorial design was utilized concerning with 3 variables at 2 levels for formulations. Full factorial design was used concerning with variables at 2 levels for formulations. Eight different formulations with low and high values of oil (12% and 30%)water (10%, 15%), S/C ratio (1:1, 4:1) and dexamethasone 0.1% were used for preparing of microemulsion formulations. Various microemulsions were chosen from the pseudo ternary phase diagram with 1:1, and 4:1 weight ratio of Tween 80/ Labrasol/ Capryo l 90. Dexamethasone (0.1%) was added to Labrafac Lipophile WL/ Transcutol P (10:1) as oil phase and vortexed, then adding surfactant–cosurfactant (S/C) mixture then appropriate amount of double distilled water was added to the mixture drop by drop and the MEs containing dexamethasone were obtained by stirring the mixtures at ambient temperature [11-13].

Stability assessment

The microemulsions physical stabilities were investigated regarding temperature stability and centrifugation. Microemulsions were stored at different temperatures (4, 25 and 37 ºC) and checked for phase separation, flocculation or precipitation. Also, Microemulsions were centrifuged by HIGH SPEED BRUSHLESS CENTERIFUGE (Vs-35sMTi, Vision) 10000 rpm for 30 minute at 25 ºC and inspected for any change in their homogeneity [14].

Animal study

250-300 g male adult Wistar rats, aged 10-12 weeks were prepared from Animals Laboratory, Jundishapur University of Medical Sciences Ahvaz, Iran. The hair on the abdominal skin was removed with an electric clipper, taking care not to damage the skin. The rats were anaesthetized with ether; their abdominal skin was shaved and then sacrificed. Abdominal full-thickness skin was removed and any extraneous subcutaneous fats cleaned from the dorsal side using cooled (4 ºC) pure acetone solution. Whole skin thickness was determined using a digital micrometer (AAOC, France)[15].

In vitro permeation study

The permeability of dexamethasone through Rat skin was investigated using Franz diffusion cells with an effective diffusion area of 3.925 cm^2. Before the experiment the frozen skins were kept at room temperature and then they were cut basis on the size of diffusion cell surface and weighted. The skin was placed on the diffusion cell and receiver and donor chambers were filled with water and remained for 16 h at room temperature, then the skins were removed, dried and their weight and thicknesses were measured. Then they were assembled between receptor and donor compartments. Volume of dexamethasone microemulsion formulation containing 5 mL was applied as donor phase and the receiver compartment which was contained 22 mL of a mixture of phosphate buffer (pH 7.4) and methanol (2:1 ratio). The receptor medium was stirred with a small magnetic bead at 200rpmcontinually.At each interval (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24, 28, 32, 48, 52 and 56h) 2mlsamples were removed from the receiver compartment and at the same time replaced with an equivalent volume of the receiver solution. Amount of permeability was performed triplicate. The permeability test of dexamethasone was determined by UV spectroscopy method at 244nm [16, 17].

Data analysis and statistics

Minitab15 software was used for experimental design and the evaluation of the variables on responses. Sigma plot11 software was applied for providing ternary phase diagrams. The cumulative amount of dexamethasone permeated per unit skin area was calculated and plotted against time. The skin permeation rate at steady state (J_{ss}) was calculated from the linear portion of the slope of the permeation curve. The one-way analysis of variance (ANOVA) was used to see any significant differences and $p<0.05$ was considered to be significant with 95% confidence intervals. All statistical analyses were conducted using SPSS software (version 16.0).All the experiments were repeated three times and data were expressed as the mean value ± SD. Different permeability parameters was measured through permeation studies that included flux(J_{ss}), permeability coefficient(P), lag time(T_{lag}) and diffusivity coefficient(D). Since the skin thickness (h)

does not show the real pathway for drug permeation then diffusivity coefficient is defined as apparent D. P and D parameters were calculated from equation(J_{ss}=P.C_0) and (D= h^2/T_{lag}), respectively. The enhancement ratio(ER) was calculated to find the relative enhancement in the permeability parameters amount of microemulsion formulations in respect of the control (drug saturated solution) permeability parameters. The enhancement ratio was estimated according to equation 1.

$$ER = \frac{\text{permeability parameter of formulation}}{\text{permeability parameter of control}}$$ -----(Equation 1)

RESULTS AND DISCUSSION

To formulate ME system for transdermal delivery of dexamethasone, suitable oil, surfactant and cosurfactant have to be chosen. Since only the drug dissolved can permeate through the skin, the solubility of poorly water soluble dexamethasone needs to be increased. It has been previously reported that the maximum solubility of dexamethasone was in labrafac lipophile WL: Transcutol P (10:1) (9.21 ± 0.13 mg/ mL) as compared to other oil mixtures. Also, the highest drug solubility of dexamethasone in surfactants is found in Labrasol (8.49 ± 0.13 mg/mL), Tween80 (6.27±0.12 mg/mL) and cosurfactant, capryol 90 (9.93±0.27 mg/mL). Based on the former solubility studies of dexamethasone in oil, surfactant and cosurfactant and the pre-formulation experiments, it was found that in labrafac lipophile WL :Transcotol P, Labrasol, Tween 80 and Capryol 90 could be the most suitable combination for preparation of microemulsion. As shown in table 1, the mean particle size of formulations was between 5.06 and 159 nm. The ME-8 formulation had the lowest average particle size 5.06±0.75 nm with polydispersity index (PDI) of 0.365±0.18 (Table 1).

Table-1: Compositions of Selected Microemulsions (% w/w) and Particle Size (mean ± SD, n=3)

Formulation	Factorial	S/C	% Oil	% (S+C)	% Water	Particle size (nm)	Polydispersity
ME-1	+ + +	4:1	30	55	15	11.36±0.6 nm	0.363±0.25
ME-2	+ + -	4:1	30	60	10	16.3±0.7 nm	0.358±0.22
ME-3	+ - +	4:1	12	73	15	14.13±0.51 nm	0.363±0.1
ME-4	+ - -	4:1	12	78	10	159±3.6 nm	0.366±0.2
ME-5	- ++	1:1	30	55	15	7.16±0.96 nm	0.363±0.1
ME-6	- +-	1:1	30	60	10	9.4±0.96. nm	0.353±0.1
ME-7	- - +	1:1	12	73	15	6.4±0.75 nm	0.363±0.2
ME-8	- - -	1:1	12	78	10	5.06±0.75 nm	0.363±0.18

***In-vitro* Skin Permeation Parameters**

The permeability parameters of various microemulsions of dexamethasone are indicated in table 2. Among the ME formulations tested, ME 5 showed the highest skin permeability. ME-5 was composed of 0.1% dexamethasone, 30% labrafac lipophil wl: transcutol P, 55% larasol-tween80/capryol 90 (1:1) and 15% water. The J_{ss} of dexamethasone from ME-5 was 0.017433±0.002892mg cm^{-2} h^{-1}, 6.011 times higher than those of the dexamethasone saturated solution in water, which were 0.0029± 0.0004 $mg.cm^{-2}.h^{-1}$. According to the results obtained from permeation studies, it was obvious that the maximum of P and T_{lag} parameters are belongs to ME-3 with 0.0795 $cm.h^{-1}$, and ME-2, 16.08 h, respectively. The maximum of D_{app} parameter was obtained from ME-5 was 0.121287±0.0187036 h^{-1}, about 60 times higher than those of the dexamethasone saturated solution in water, which were 0.0019892±0.000212 $cm.h^{-1}$.To compare the results, multivariate regression was applied for the analysis of correlation between independent variables and MEs skin permeability parameters. Analysis of variance showed no significant differences between independent variables and J_{ss}, P, T_{lag} and D_{app} parameters of dexamethasone formulations ($p>0.05$).

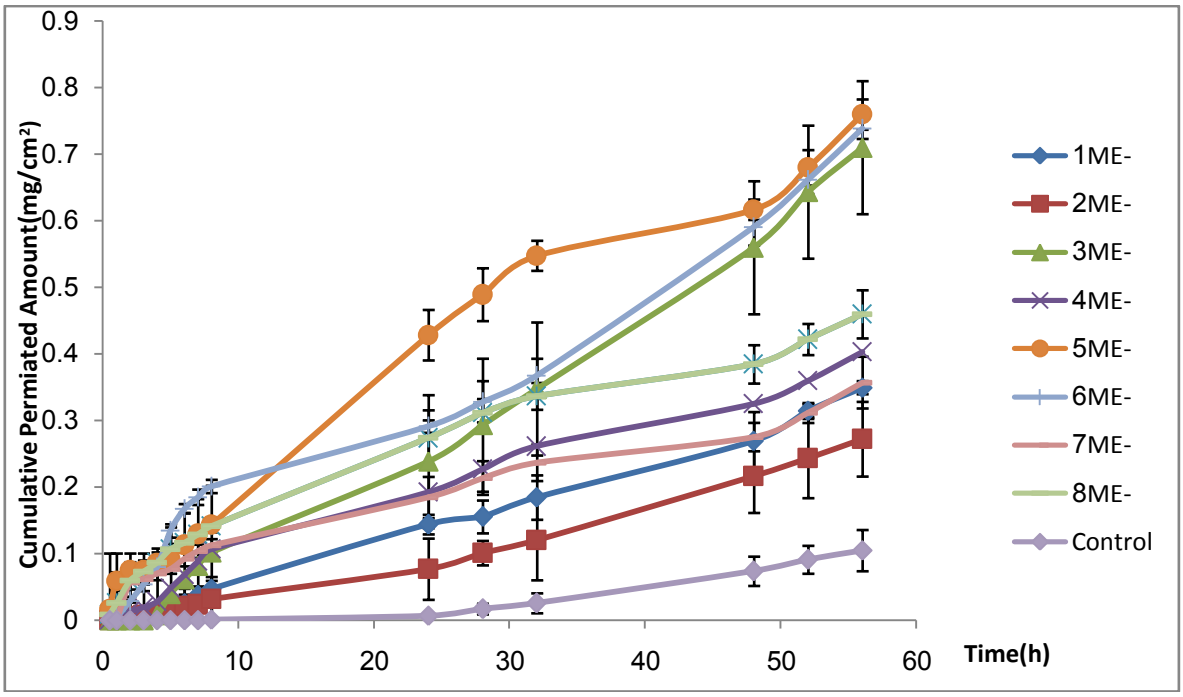

Figure 2. Permeation profiles of dexamethasone through excised rat skins from various microemulsions

Table 2: *In vitro* permeability parameters of dexamethasone aqueous saturated solution(control) and various ME formulations through excised rat skins(mean±SD, n=3)

Formulation	J_{ss} (mg/cm^2.h)	T_{lag}(h)	D_{app}(cm^2/h)	P(cm/h)	ER_{flux}	ER_D
Control	0.0029± 0.0004	22.896907± 5.432001	0.0019892 ±0.000212	2.7396792 ±0.812024	-	-
ME-1	0.0098± 0.005656	4.322729± 1.235268	0.017577± 0.010879	0.009766± 0.005617	3.37931± 1.950335	8.836216± 5.469287
ME-2	0.007533± 0.000764	16.08854± 4.687767	0.003178± 0.000833	0.007505± 0.000751	2.597701± 0.263366	1.596957± 0.417415
ME-3	0.0185± 0.003913	3.784767± 0.8045839	0.017722± 0.00979	0.079529± 0.0107535	6.37931± 1.349241	8.894809± 4.899092
ME-4	0.015367± 0.005811	3.603147± 0.3185298	0.023553± 0.021403	0.015093± 0.005433	5.298851± 2.003663	11.84061± 10.7617
ME-5	0.017433± 0.002892	2.820184± 2.686382	0.121287± 0.0187036	0.017346± 0.002851	6.011494± 0.997222	60.97275± 21.722031
ME-6	0.032367± 0.002139	1.469491± 0.525519	0.038318± 0.013067	0.032261± 0.002182	11.16092± 0.737426	19.26235± 6.568714
ME-7	0.008267± 0.002994	10.52403± 3.45718	0.014518± 0.014114	0.008236± 0.002969	2.850575± 1.032373	7.298244± 7.095227
ME-8	0.0132± 0.005103	4.520927± 1.233516	0.091156± 0.012969	0.008635± 0.002945	4.551724± 1.759635	45.82579± 39.19701

CONCLUSION

The results of the present study showed that any change in component and ratio of oil phase, surfactant and cosurfactant may change the permeability parameters during drug permeation from microemulsions. The phenomenon may be due to alteration of solubility of drug, partitioning between aqueous and lipid phase, and also structural changes of the membrane in the presence of microemulsion components. It can be concluded that a lower surfactant/cosurfactant ratio, improves the most permeability parameters of dexamethasone microemulsion.

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CONFLICT OF INTERESTS

Authors have declared that no competing interests exist.

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