

ACCELERATED STABILITY TESTING OF BETAMETHASONE DIPROPIONATE NANOEMULSION

MD. SARFARAZ ALAM^{1*}, SANJULA BABOOTA², MD. SAJID ALI¹, MAKSOOD ALI¹, NAWAZISH ALAM¹, MD. INTAKHAB ALAM¹, JAVED ALI².¹College of Pharmacy, Jazan University, Jazan, KSA, ²Department of Pharmaceutics, Faculty of Pharmacy, Jamia Hamdard, New Delhi-110062, India. Email: sarfarazpharma1981@gmail.com

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ABSTRACT

The nanoemulsion formulation containing Betamethasone dipropionate (BD) was prepared by spontaneous emulsification method. These were characterized by droplet size, viscosity and refractive index. Stability studies were performed for a period of 3-months. Droplet size, viscosity and refractive index were determined every month for three months. Shelf-life of nanoemulsion formulation was also determined after performing the accelerated stability testing. It was found that the droplet size, viscosity and refractive index were slightly increased at refrigerator (4°C) and room temperature (25°C) during 3-months of storage period. However, the changes in these parameters were not statistically significant ($p > 0.05$). The shelf-life of optimized nanoemulsion formulation was found to be 2.66 years at room temperature. These results indicated that the stability (physical and chemical) of BD was enhanced in nanoemulsion formulation.

Keywords: Nanoemulsion, Betamethasone dipropionate, Shelf- life, Stability.

INTRODUCTION

Betamethasone dipropionate (BD) is a highly potent glucocorticoid receptor agonist which possesses immunosuppressive, anti-inflammatory, and anti-proliferative effects. It exerts its action by inhibition of phospholipase A₂ which leads to the inhibition of synthesis of arachidonic acid and controls the biosynthesis of prostaglandins and leukotrienes. There are various types of dosage forms available including ointment, cream, lotion and foam which are used for the treatment of mild to moderate psoriasis (1-3). However, the clinical limitation of BD has poor permeability through skin which reduces its therapeutic effectiveness at the target site. The main limitation lies in the barrier function of the skin. Therefore, the major challenges for a topical formulation are to provide a sufficient increase in drug penetration into the skin, without any significant functional and histological change in the skin and also irreversible alteration to the skin barrier function (4).

In recent years, much attention has been focused on lipid-based formulations to improve the permeability and bioavailability of poorly water soluble drug compounds. Many of the dermal vehicles contain chemical enhancers and solvents to achieve these goals. But the use of chemical enhancers may be harmful, especially in chronic application, as many of them are irritants. Therefore, natural oils such as oleic acid are selected which act as a penetration enhancer as well as vehicle for the nanoemulsion. Nanoemulsions are thermodynamically stable, transparent dispersions of oil and water stabilized by an interfacial film of surfactant and co-surfactant molecules having the droplet size 10 - 200 nm (5-7). Nanoemulsions have been known to increase therapeutic efficacy of many drugs. Nanoemulsions have been known to enhance the physical as well as chemical stability of many drugs (8). Therefore, attempts were made in present study to enhance physical as well as chemical stability of BD using nanoemulsion formulation.

MATERIALS AND METHODS

Materials

BD was obtained as a gift sample from Gaurav Pharma Limited (Delhi, India). Oleic acid was obtained from Mahaveer Enterprises (Rajasthan, India). Eucalyptus oil was obtained from Scientific International (New Delhi). PEG 200, Tween 80, Tween 20, Pleurol oleic, Glycol, Brij35, Propanol, Isopropyl alcohol and ethanol were purchased from Merck (Merck, India). Labrasol, Safsol and capryol were obtained as a kind gift samples from Gattefosse (Mumbai, India). All other chemicals used were of analytical grade.

Preparation of betamethasone dipropionate nanoemulsion

Nanoemulsions of BD were prepared by spontaneous emulsification method. Detail description of their preparation, characterization and

optimization is given in our published article (9). Optimized nanoemulsion was prepared by dissolving 0.05% (w/v) of BD in 15% (v/v) mixture of oleic acid and safsol (1.5:1 v/v). Then 38% (v/v) mixture of Tween- 20 and Isopropyl alcohol (1:1 v/v) were added slowly in oil phase. Then remaining amount of distilled water was added slowly to get the final preparation of 100% (v/v) (9-11).

Characterization of nanoemulsion

Droplet size and its distribution was determined by photon correlation spectroscopy (PCS), using a Zetasizer 1000 HS (Malvern Instruments, UK). Light scattering was monitored at 25°C at a scattering angle of 90°.

The viscosity of the nanoemulsion was determined using Brookfield DV III ultra V6.0 RV cone and plate rheometer (Brookfield Engineering Laboratories, Inc, Middleboro, MA) using spindle # CPE40 at 25 ± 0.3°C (12-13).

Refractive index (RI) of nanoemulsion formulation was determined using an Abbes type refractometer (precision standard testing equipment corporation, India).

Stability studies as per ICH guidelines

Accelerated stability studies were also performed on optimized BD nanoemulsion as per international conference on harmonization (ICH) guidelines. Stability studies on optimized nanoemulsion were performed by keeping the sample at refrigerator temperature (4°C) and room temperature (25°C). These studies were performed for the period of 3 months. The droplet size, viscosity and refractive index were determined at 0, 1, 2 and 3 months. Three batches of optimized formulation were taken in glass vials and were kept at accelerated temperature of 30, 40, 50 and 60°C at ambient humidity. The samples were withdrawn at regular intervals of 0, 1, 2 and 3 months. A reversed-phase high performance liquid chromatography (RP-HPLC) method was used for determination of BD. The HPLC column was the YMC J'sphere ODS-H80, 150 mm × 4.6 mm I.D., 4 μm particle size (YMC, Milford, MA). The mobile phases consisted of A: 0.05% (v/v), methanesulfonic acid in water and B: 100% acetonitrile in (70/30, v/v). The flow rate was 2.0 mL/min and column temperature was 35 °C. Retention time was found to be 4.2 min. Analysis was carried out at each time interval by taking 100 μl of each formulation and diluting it to 5 ml with methanol and injecting into the HPLC system at 240 nm. The pH of the sample was adjusted to about 2.7 by 1 M hydrochloric acid. The solubility of sample in methanol was 43.6 mg/ml. In addition, samples of pure oil (combination of Safsol 218 and oleic acid), pure surfactant, cosurfactant and S_{mix} were run separately to check interference of the excipients used in the formulations (14).

The amount of drug decomposed and the amount remaining (undecomposed drug) at each time interval was calculated. Order of degradation was determined by the graphical method (15). Degradation rate constant (K) was determined at each temperature. Arrhenius plot was constructed between log K and 1/T to determine the shelf-life of optimized nanoemulsion formulation. The degradation rate constant at 25°C (K₂₅) was determined by extrapolating the value of at 25°C from Arrhenius plot. The shelf-life (T_{0.9}) for formulation was determined by using the formula:

$$\text{Shelf Life} = \frac{0.1052}{K}$$

Where, K is the degradation rate constant.

RESULTS AND DISCUSSION

Stability of a drug product refers to the chemical and physical integrity of the dosage unit and when appropriate, the ability of the drug product to maintain protection against microbiological contamination. An ideal drug product must be fully characterized physically, chemically and microbiologically at the start of study and throughout the intended shelf-life period. Therefore optimized nanoemulsion formulation was characterized for droplet size, viscosity and RI for the period of three months. These parameters were determined at 0, 1, 2 and 3 months. It was found that at 4 and 25°C these parameters were slightly increased during storage at both the temperatures (Table 1).

These parameters were compared for statistical significance by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test using Graph Pad In stat software (GraphPad Software Inc., CA, USA). The changes in these parameters

were not statistically significant ($P \geq 0.05$). These results indicated that the optimized formulation was stable as there were no significant changes in physical parameters (droplet size, viscosity and RI). The degradation of BD was very slow at each temperature which indicated the chemical stability of BD in the nanoemulsion formulation. The optimized nanoemulsion was found to be stable chemically as well as physically.

The degraded and remained concentrations of BD at different temperatures are shown in Table 2. The order of degradation was determined by graphical method at each temperature. The order of degradation was found to be first order (Fig. 1). The rate of degradation is directly proportional to the first power of the concentration of a single reactant in first order degradation. The correlation coefficients of first order degradation as well as zero order degradation were determined at each temperature as shown in Fig. 1 and 2. Therefore for first order degradation, Log % of drug remaining was plotted against time (Fig. 1) and K was calculated from the slope of the curve at each temperature by following formula:

$$\text{Slope} = \frac{-K}{2.303}$$

Where, K is the degradation rate constant

The values of K at each temperature are given in the Table 3. The log of drug remaining was plotted against time (months). Slope of each line was obtained and K was calculated by the formula. The effect of temperature on the degradation was studied by plotting log K v/s 1/T. (Fig. 3). The value of K at 25°C (K₂₅) was obtained by extrapolation of the plot and shelf-life was then calculated. The shelf-life of optimized nanoemulsion formulation was found to be 2.66 years.

Table 1: Droplet size, viscosity and RI of optimized nanoemulsion A2 during storage

Time (months)	Temperature (°C)	Mean droplet size (nm) ± SD (n=3)	Mean Viscosity (mP) ± SD (n=3)	RI ± SD (n=3)
0	4.0 ± 0.5	114.00 ± 1.73	28.55 ± 1.62	1.409 ± 0.023
1	4.0 ± 0.5	114.33 ± 1.76	28.57 ± 1.71	1.410 ± 0.020
2	4.0 ± 0.5	114.65 ± 1.83	28.94 ± 1.53	1.414 ± 0.021
3	4.0 ± 0.5	115.15 ± 1.89	28.99 ± 1.61	1.416 ± 0.027
0	25 ± 0.5	114.00 ± 1.73	28.55 ± 1.62	1.409 ± 0.023
1	25 ± 0.5	114.39 ± 1.93	28.71 ± 1.71	1.412 ± 0.028
2	25 ± 0.5	115.07 ± 2.31	28.83 ± 1.39	1.417 ± 0.025
3	25 ± 0.5	115.46 ± 2.47	29.19 ± 1.37	1.419 ± 0.021

Table 2: Degradation of optimized nanoemulsion A2

Time (Days)	Temperature (°C)	Drug content (mg)	Drug concentration degraded (mg)	% drug remaining	Log % drug remaining
0	30 ± 0.5	50	0	100	2
30	30 ± 0.5	49.77	0.23	99.54	1.997998
60	30 ± 0.5	49.087	0.913	98.174	1.991996
90	30 ± 0.5	48.749	1.251	97.498	1.988996
0	40 ± 0.5	50	0	100	2
30	40 ± 0.5	49.695	0.305	99.396	1.99737
60	40 ± 0.5	48.749	1.251	97.498	1.988996
90	40 ± 0.5	48.0805	1.9195	96.161	1.982999
0	50 ± 0.5	50	0	100	2
30	50 ± 0.5	49.087	0.913	98.174	1.991996
60	50 ± 0.5	48.637	1.363	97.274	1.987997
90	50 ± 0.5	47.6395	2.3605	95.279	1.978997
0	60 ± 0.5	50	0	100	2
30	60 ± 0.5	49.3135	0.6865	98.627	1.993996
60	60 ± 0.5	48.191	1.809	96.382	1.983996
90	60 ± 0.5	47.2005	2.7995	94.401	1.974977

Table 3: Observation table for calculation of shelf life of nanoemulsion A2

Temperature (°C)	Slope	K (month ⁻¹)	Log K	Absolute Temperature (T)	1/T × 10 ³
30	-0.003	0.006909	-2.16058	303	3.30033
40	-0.005	0.011515	-1.93874	313	3.19488
50	-0.006	0.013818	-1.85955	323	3.09597
60	-0.008	0.018424	-1.73462	333	3.00300
25		0.00476	-2.3216	298	3.35570

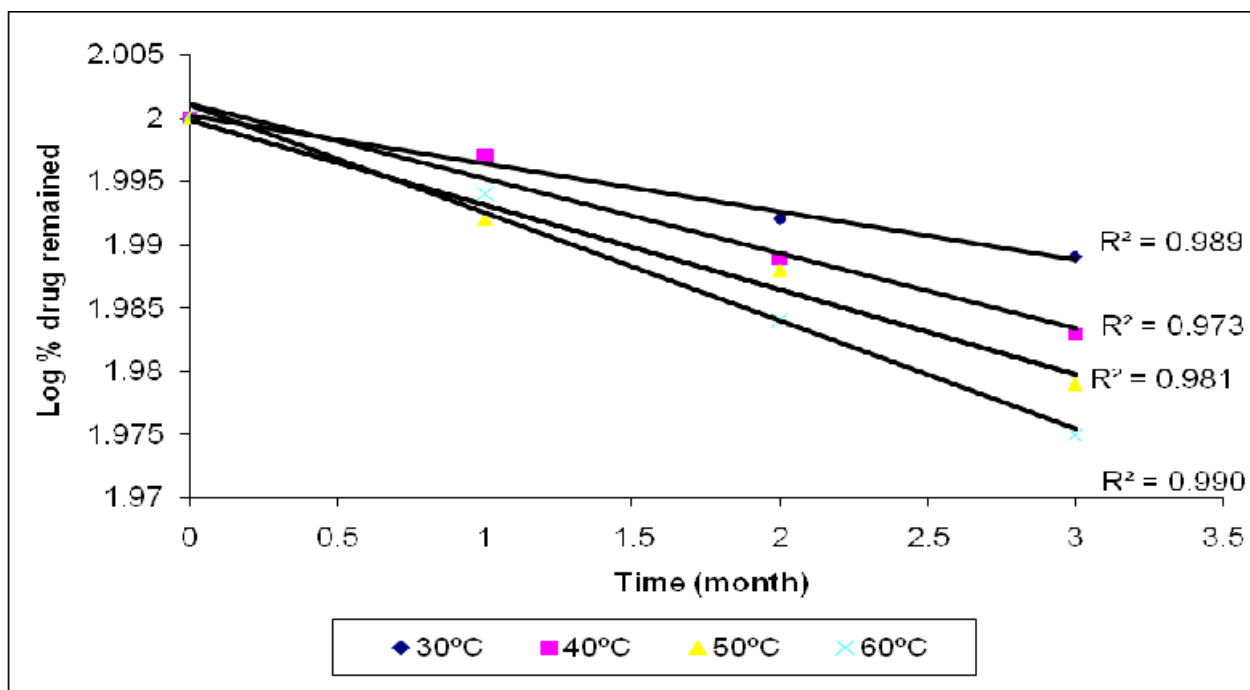


Fig. 1: First order degradation kinetics of BD from nanoemulsion formulation at different temperatures.

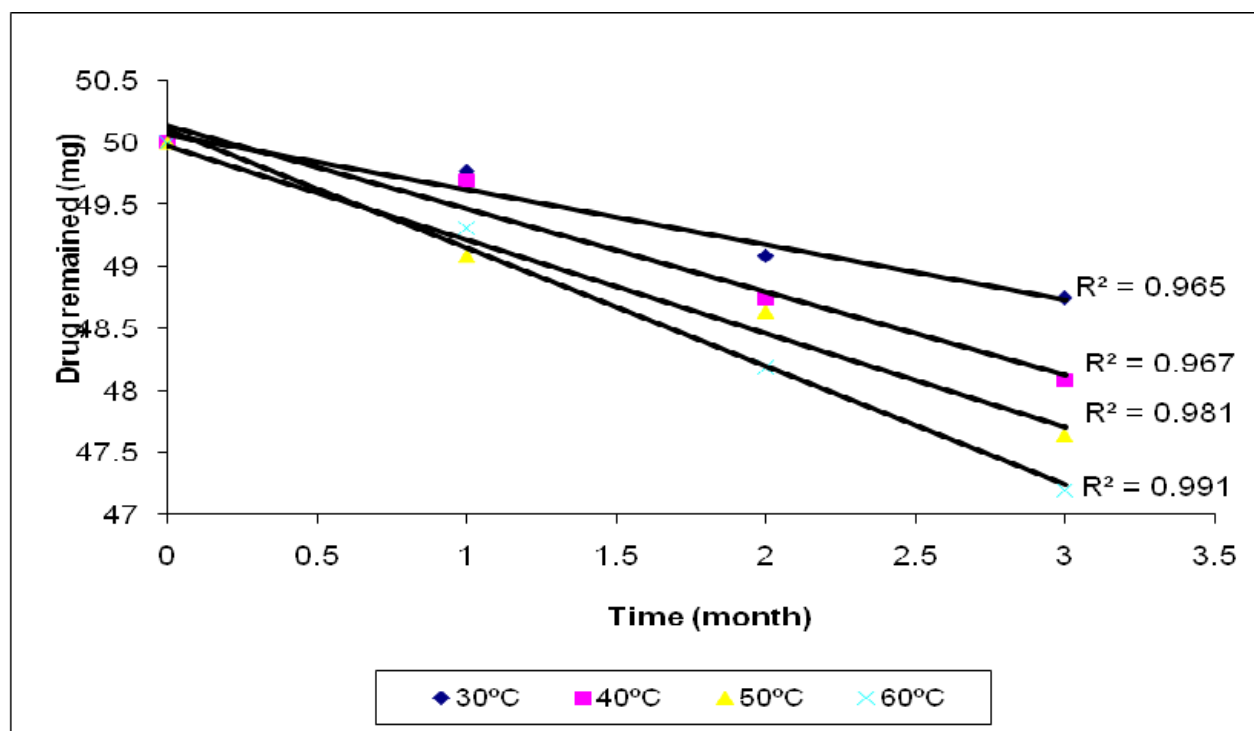


Fig. 2: Zero order degradation kinetics of BD from nanoemulsion formulation at different temperatures

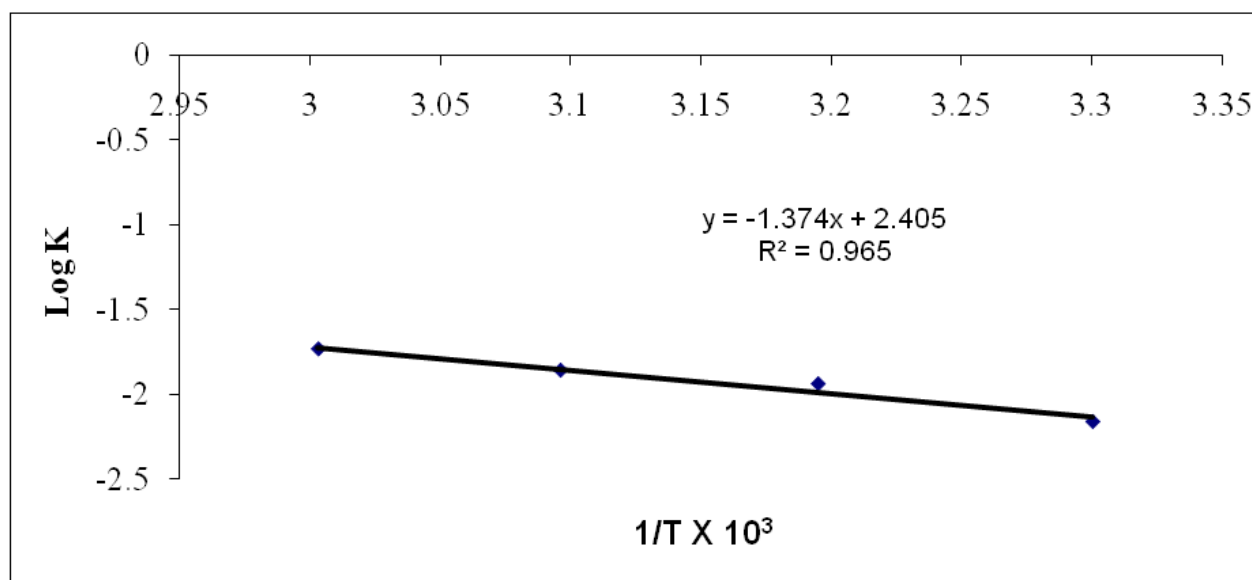


Fig. 3: Arrhenius plot between Log K and 1/T for nanoemulsion formulation A2

CONCLUSION

The droplet size, viscosity and RI of optimized nanoemulsion formulation were not significantly changed during 3 months of storage period suggesting the physical stability of the prepared nanoemulsion. The degradation of BD after 3 months of storage was found to be lowest in the formulation. Slower degradation of BD indicated the chemical stability of BD in nanoemulsion. The shelf-life of nanoemulsion formulation was found to be 2.66 years at room temperature (25°C). For the study it can be concluded that the stability (Physical and chemical) of BD can be enhanced by incorporating in nanoemulsion.

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