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Full Length Research Paper

Accelerated stability testing of celecoxib nanoemulsion containing Cremophor-EL

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Celecoxib (CXB), a selective cyclooxygenase-2 inhibitor has been recommended for the treatment of arthritis and osteoarthritis upon oral administration. However, long term oral administration of celecoxib cause serious gastrointestinal adverse effects. Therefore the aim of the present study was to enhance CXB's physical and chemical stability using nanoemulsion formulation in order to eliminate gastrointestinal adverse effects of its oral administration. Optimized nanoemulsion formulation was prepared by spontaneous emulsification method. Nanoemulsion was characterized by droplet size, viscosity and refractive index. Stability studies were performed for the period of 3 months. Droplet size, viscosity and refractive index were determined every month. Shelf- life of nanoemulsion formulation was also determined by accelerated stability testing. It was found that droplet size, viscosity and refractive index were slightly increased at refrigerator and room temperature in 3 months 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 2.38 years at room temperature. These results indicated that both physical as well as chemical stability of celecoxib can be enhanced in nanoemulsion formulation using Cremophor-EL as surfactant.

Key words: Nanoemulsion, celecoxib, shelf life, cremophor-EL.

INTRODUCTION

Celecoxib (CXB), a selective cyclooxygenase-2 (COX -2) inhibitor has been recommended for the treatment of arthritis and osteoarthritis upon oral administration (Shakeel et al., 2008b). However long-term oral administration of CXB causes serious gastrointestinal (GI) adverse effects (Baboota et al., 2007a). Therefore, CXB was formulated into nanoemulsion formulation for transdermal drug delivery in order to eliminate its GI adverse effects (Shakeel et al., 2009). 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 - 100 nm (Baboota et al., 2007a; Shafiq et al., 2007a; Shakeel et al., 2008a; Shakeel et al., 2008c). Nano-emulsions have been known to increase therapeutic efficacy of many drugs (Baboota et al., 2007a; Shakeel et al., 2007; Kuo et al., 2008; Tagne et al., 2008). Nano-emulsions have a higher solubilization capacity than simple micellar solutions and their thermodynamic stability offers advantages over unstable dispersions, such as emulsions and suspensions (Baboota et al., 2007b; Shafiq et al., 2007b, c) . Moreover they can be manufactured with little energy input (heat or mixing) and have long shelf-life (Baboota et al., 2007b). Nano-emulsions prepared by spontaneous emulsification method present many advantages over other drug carriers, namely lower preparation cost, good production feasibility, higher storage stability, thermodynamic stability, absence of organic solvents and no need of intensive fornication (Shakeel et al., 2009). 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 (Ahmed et al., 2008). Stability of drug product or dosage form is one of

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of the problems associated in the development of liquid dosage forms like suspensions, emulsions, microemulsions and nanoemulsions. Nanoemulsions have been known to enhance the physical as well as chemical stability of many drugs with no adverse effects such as GI complications (Baboota et al., 2007a, c; Shafiq et al., 2007a). Therefore, attempts were made in present study to enhance physical as well as chemical stability of CXB using nanoemulsion formulation.

MATERIALS AND METHODS

Materials

Celecoxib was a kind gift sample from Ranbaxy Research Labs (India). Propylene glycol mono caprylic ester (Sefsol 218) was obtained as gift sample from Nikko Chemicals (Japan). Diethylene glycol monoethyl ether (Transcutol-P) was gift sample from Gattefosse (France). Glycerol triacetate (Triacetin) was purchased from E-Merck (India). Polyoxy- 35-castor oil (Cremophor-EL) was purchased form Sigma Aldrich, USA. All other chemicals used in the study were of analytical reagent (AR) grade.

Preparation of CXB nanoemulsion

Various nanoemulsions of CXB were prepared by spontaneous emulsification method. Detail description of their preparation, characterization and optimization is given in our forthcoming article (Shakeel et al., 2009). Optimized nanoemulsion was prepared by dissolving 2% w/w of CXB in 15% w/w mixture of Sefsol-218 and Triacetin (1:1). Then 35% w/w mixture of Cremophor-EL and Transcutol- P (1:1) were added slowly in oil phase. Then remaining amount of distilled water was added slowly to get the final preparation 100% w/w (Shakeel et al., 2007; Shakeel et al., 2009).

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 (Baboota et al., 2007a; Shafiq et al., 2007a; Shakeel et al., 2007).

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

Stability studies as per ICH guidelines

Stability studies on optimized nanoemulsion were performed by keeping the sample at refrigerator temperature (4oC) and room temperature (25oC). 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. Accelerated stability studies were also performed on optimized CXB nanoemulsion as per international conference on harmonization (ICH) guidelines. Three batches of optimized formulation were taken in glass vials and were kept at accelerated temperature of 30, 40, 50 and 60oC at ambient humidity. The samples were withdrawn at regular intervals of 0, 1, 2 and 3 months. These samples were analyzed for drug content by stability-indicating HPLC method at a wavelength of 250 nm (Baboota et al., 2007c). The chromatographic column used was a reverse phase 25 cm X 4.6 mm, i.d., 5 m, 516 C18 DB reversed phase column (Supelco). The mobile phase was methanol: water (75:25) with the flow rate of 1.25 ml/min. The retention time (Rt) of CXB was 4.8 min.Zero time samples were used as controls (100% drug) . 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 250 nm. The solubility of sample in methanol was 115.6 mg/ml. In addition, samples of pure oil (combination of Sefsol 218 and Triacetin), pure surfactant and cosurfactant (S_{mix}) were run separately to check interference of the excipients used in the formulations.

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 (Shafiq and Shakeel, 2008). 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^{o}C (K_{25}) was determined by extrapolating the value of 25^{o}C from Arrhenius plot. The shelf-life ($T_{0.9}$) for each formulation was determined by using the formula:

$$T_{09} = \frac{0.1052}{K_{25}}$$

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. During stability studies droplet size, viscosity and RI were determined at 4 and 25°C. These parameters were determined at 0, 1, 2 and 3 months. It was found that droplet size, viscosity and RI were slightly increased in time at both 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 GraphPad Instat software (GraphPad Software Inc., CA, USA). The changes in these parameters were not statistically significant (P 0.05). These results indicated that optimized formulation is stable as there were no significant changes in physical parameters (droplet size, viscosity and RI). For accelerated stability studies, samples were withdrawn at regular intervals of 0, 1, 2, and 3 months. The samples were analyzed for their drug content by HPLC analysis at a wavelength of 250 nm.

The degradation of CXB was very slow at each temperature which indicated the chemical stability of CXB in the nanoemulsion formulation. The optimized nanoemulsion was found to be stable chemically as well as physically, it was concluded that it is suitable for transdermal delivery

Table 1. Droplet size, viscosity and RI of optimized nanoemulsion formulation during storage.

Time (months)	Temperature (°C)	Droplet size mean ± SD (nm)[a]	Viscosity mean ± SD (cps)[a]	RI ± SD[b]
0	4.0 ± 0.5	16.41 ± 1.72	18.47 ± 1.78	1.399 ± 0.006
1	4.0 ± 0.5	16.52 ± 1.81	18.82 ± 1.91	1.401 ± 0.008
2	4.0 ± 0.5	16.61 ± 1.87	18.96 ± 2.19	1.402 ± 0.009
3	4.0 ± 0.5	16.89 ± 1.99	19.11 ± 2.43	1.404 ± 0.011
0	25 ± 0.5	16.41 ± 1.72	18.47 ± 1.78	1.399 ± 0.006
1	25 ± 0.5	16.59 ± 1.88	18.96 ± 2.15	1.401 ± 0.009
2	25 ± 0.5	16.86 ± 2.21	19.13 ± 2.36	1.403 ± 0.012
3	25 ± 0.5	17.13 ± 2.58	19.44 ± 2.42	1.405 ± 0.013

[a]Mean ± SD, n = 3, [b]Mean ± SD, n = 6

Table 2. Degradation of optimized nanoemulsion formulation.

Time (months)	Temperature (°C)	Concentration found (mg)	Concentration degraded (mg)	% remained	Log % remained
0	30 ± 0.5	20.00	0.00	100.00	2.00
1	30 ± 0.5	19.93	0.07	99.65	1.9984
2	30 ± 0.5	19.90	0.10	99.50	1.9978
3	30 ± 0.5	19.85	0.15	99.25	1.9967
0	40 ± 0.5	20.00	0.00	100.00	2.00
1	40 ± 0.5	19.88	0.12	99.40	1.9973
2	40 ± 0.5	19.82	0.18	99.10	1.9960
3	40 ± 0.5	19.78	0.22	98.90	1.9951
0	50 ± 0.5	20.00	0.00	100.00	2.00
1	50 ± 0.5	19.80	0.20	99.00	1.9956
2	50 ± 0.5	19.66	0.34	98.30	1.9925
3	50 ± 0.5	19.47	0.53	97.35	1.9883
0	60 ± 0.5	20.00	0.00	100.00	2.00
1	60 ± 0.5	19.69	0.31	98.45	1.9932
2	60 ± 0.5	19.38	0.62	96.90	1.9863
3	60 ± 0.5	19.24	0.76	96.20	1.9831

The degradation of CXB was very slow at each temperature which indicated the chemical stability of CXB in the nanoemulsion formulation. The optimized nanoemulsion was found to be stable chemically as well as physically, it was concluded that it is suitable for transdermal delivery of CXB. Transdermal potential of this formulation has already been proved in our forthcoming article (Shakeel et al., 2009).

The degraded and remained concentration of CXB at different temperatures is 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 (Figure 1). In first order degradation, the rate of degradation is independent of the concentration of reacting species. However, 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 were significant as compared to correlation coefficients of zero order degradation at each temperature as shown in Figure 1 and 2 ($p < 0.05$).

Therefore for first order degradation, Log % of drug remaining was plotted against time (Figure 1) and K was calculated from the slope of the curve at each temperature.

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

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

The effect of temperature on the degradation was studied by plotting log K v/s 1/T. (Figure 3). The value of K at 25°C (K_{25}) was obtained by extrapolation of the plot and shelf-life was then calculated. The shelf-life of optimized

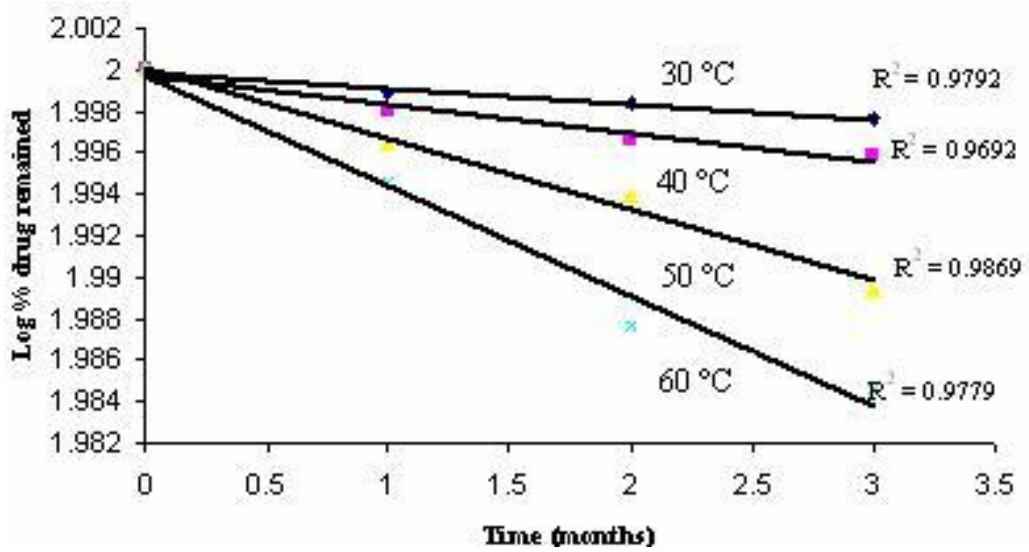

Figure 1. First order degradation kinetics of CXB from nanoemulsion formulation at different temperatures.

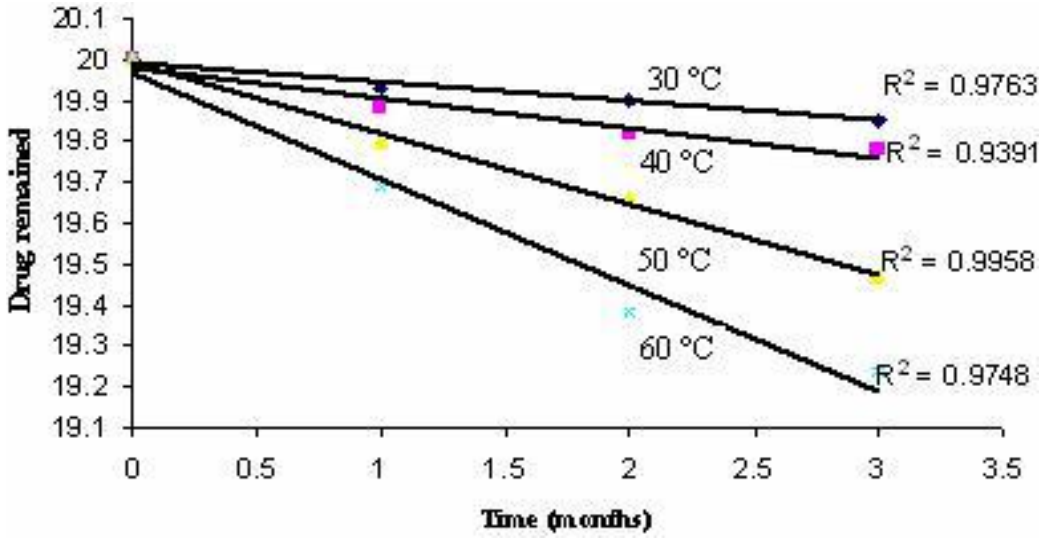

Figure 2. Zero order degradation kinetics of CXB from nanoemulsion formulation at different temperatures.

Table 3. Observation table for calculation of shelf life of nanoemulsion formulation.

Temperature	Slope	K x 10^{-3} ($month^{-1}$)	Log K	Absolute Temperature	1/T x10^{3}
30	-0.0011	2.533	-2.5963	303.00	3.300330
40	-0.0016	3.6848	-2.4335	313.00	3.194488
50	-0.0038	8.7514	-2.0579	323.00	3.095975
60	-0.0058	13.3574	-1.8742	333.00	3.003003
25		3.6719	-2.4351	298.00	3.355704

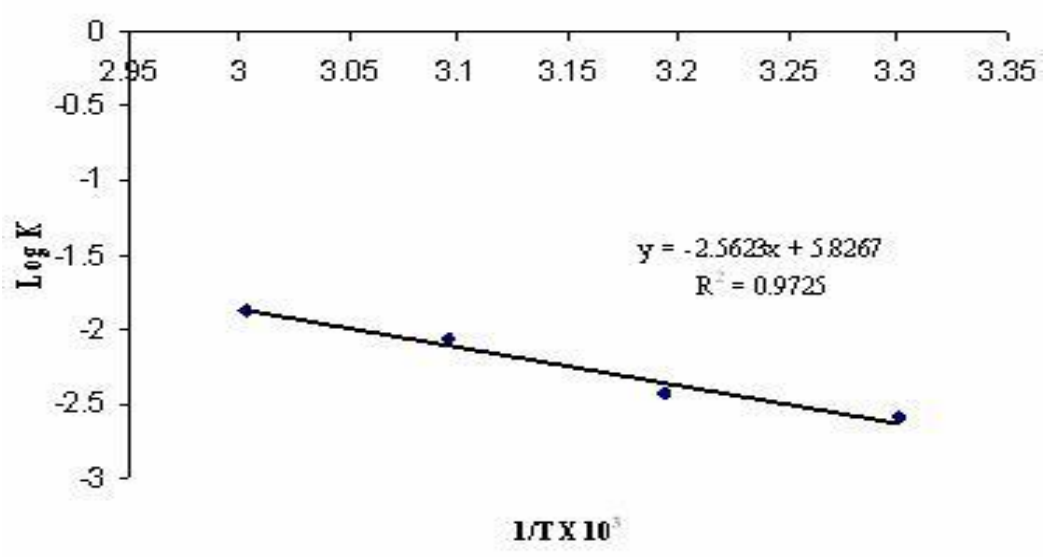

Figure 3. Arrhenius plot between Log K and 1/T for nanoemulsion formulation.

nanoemulsion formulation was found to be 2.38 years.

Conclusion

The droplet size, viscosity and RI of optimized nanoemulsion formulation were not significantly changed during 3 months of storage suggesting that prepared nanoemulsion was physically stable. The degradation of CXB after 3 months of storage was also slowest in the formulation. Slower degradation of CXB indicated the chemical stability of CXB in nanoemulsion. The shelf-life of nanoemulsion formulation was found to be 2.38 years at room temperature. These results indicated that both physical as well as chemical stability of CXB can be enhanced in nanoemulsion formulation using Cremophor-EL as surfactant.

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