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Stability evaluation of tamoxifen citrate nanoemulsion containing Cremophor RH 40 as surfactant

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Abstract

The aim of the present study was to evaluate the stability of tamoxifen citrate using novel nanoemulsion formulation. Optimized nanoemulsion formulation of tamoxifen citrate was prepared by spontaneous emulsification method (aqueous titration method). Stability studies were performed for the period of 3 months. Droplet size, viscosity and refractive index (RI) were determined during storage. Shelf life of nanoemulsion formulation was also determined by accelerated stability testing. It was found that droplet size viscosity and RI were slightly increased at refrigerator and room temperature. The changes in these parameters were not significant ($p < 0.05$). The shelf life of nanoemulsion formulation was found to be 2.05 years at room temperature. These results indicated that stability of tamoxifen citrate can be enhanced in nanoemulsion formulation using cremophor RH 40 as surfactant.

Keyword: tamoxifen citrate, nanoemulsion, stability, Cremophor RH 40

Introduction

Tamoxifen citrate (TAM), an estrogen receptor antagonist is known to be a drug of choice for hormone sensitive breast cancer (Clarke et al. 2003). Tamoxifen is generally administered through oral and parenteral route. Despite being quite effective on oral administration, TAM exhibits certain side effects like distaste for food, abdominal cramps, nausea and vomiting. However, its other infrequent side effects include endometrial carcinoma, ocular problems, thromboembolic disorders and acquired drug resistance on long-term therapy (Morrow and Jordan 1993, Jordan 1995, Brigger et al. 2001). Tamoxifen undergoes extensive hepatic metabolism after oral administration in humans. The usual oral dose of tamoxifen is 10 mg twice daily. The steady-state plasma concentration of 77–274 ng mL^{-1} has been reported for tamoxifen (Furr and Jordan 1984). It is a widely used adjuvant therapy following surgery for breast malignancies in postmenopausal women. This agent is also indicated for treatment of estrogen receptor-positive tumors in the premenopausal population (Jordan and Murphy 1990). The recent trend for the enhancement of solubility/bioavailability is lipid based system such as microemulsions, nanoemulsions, solid dispersions, solid lipid nanoparticles and liposomes etc. This is also the most advanced approach commercially, as formulation scientists increasingly

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turn to a range of nanotechnology- based solutions to improve drug solubility and bioavailability. Nanoemulsions have been reported to make the plasma concentration profiles and bioavailability of poorly soluble drugs more reproducible. (Constantinides 1995, Lawrence and Rees 2000, Kommuru et al. 2001, Kawakami et al. 2002a and 2002b). One of the most promising techniques for enhancement of transdermal permeation of drugs is the microemulsion or nanoemulsion technique (Osborne et al. 1991, Trotta et al. 1996). Nanoemulsions are thermodynamically stable transparent (translucent) dispersions of oil and water stabilized by an interfacial film of surfactant and cosurfactant molecules having average droplet size of 10 to 140nm (Hiemenz and Rajgopalan 1997, Shafiq et al. 2007a and 2007b). Studies have shown that nanoemulsion formulations possess improved transdermal and dermal delivery properties *in vitro* (Delgado-Charro et al. 1997, Kreilgaard et al. 2000, Lee et al. 2003) and *in vivo* (Kreilgaard et al. 2000 and 2001) over emulsions (Kreilgaard 2003) and gels (Gasco et al. 1991, Kriwet and Muller-Goymann 1995). Stability of a dosage form refers to the chemical and physical integrity of the dosage unit and when appropriate, the ability of the dosage unit to maintain protection against microbiological contamination. An ideal drug product must be thoroughly characterized physically, chemically and microbiologically at the start of study and throughout the intended shelf life period (Floyd 1999). Therefore, the aim of the present study was to evaluate the stability parameters of tamoxifen citrate using nanoemulsion formulation.

Materials and Methods

Materials

Tamoxifen citrate was a gift sample from Biochem Pharmaceutical Ltd., India. Oleic acid was purchased from S.D Fine Chemicals, India. Labrafil M 1944CS was gift sample from Gattefosse, France. Cremophor RH 40, Tween 80 was gift sample from Cadila Health Care Ltd., India. Distilled water was purchased freshly from Chetak Distillery Ltd Rahuri, India. All other chemical and reagent used in the study were of analytical reagent grade.

Preparation of tamoxifen citrate nanoemulsion

On the basis of the solubility studies, oleic acid was selected as the oil phase. Cremophor RH 40 and ethanol were selected as surfactant and cosurfactant, respectively. Distilled water was used as an aqueous phase. Surfactant and cosurfactant (S_{mix}) were mixed at different mass ratios (1:1, 1:2, 2:1). These ratios were chosen in increasing concentration of surfactant with respect to cosurfactant and increasing concentration of cosurfactant with respect to surfactant for a detailed study of the phase diagrams. For each phase diagram, oil and S_{mix} at a specific ratio was mixed thoroughly at different mass ratios from 1:9 to 9:1 in different glass vials. Nine different combinations of oil and S_{mix}, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1, were made so that maximum ratios were covered for the study to delineate the boundaries of phases precisely formed in the phase diagrams. Pseudo ternary phase diagrams of oil, S_{mix} and aqueous phase were developed using the aqueous titration method. Slow titration with aqueous phase was performed for each mass ratio of oil and S_{mix} and visual observations were made for transparent and easily flowable o/w nanoemulsions. The physical state of the nanoemulsion was marked on a pseudo-three-component phase diagram with one axis representing the aqueous phase, the second one representing oil and the third representing a mixture of surfactant and cosurfactant at a fixed mass ratio.

Selection of nanoemulsion formulation

From phase diagram constructed, different formulation were selected from the nanoemulsion region were subjected to different thermodynamic stability tests. From each phase diagram constructed, different formulation were selected from the nanoemulsion region so that the drug could be incorporated into the oil phase. 5% of tamoxifen citrate, which was kept constant in all the selected formulations, was dissolved in the oil phase of nanoemulsion formulation. Selected formulations were subjected to different thermodynamic stability tests.

Thermodynamic stability studies

To overcome the problem of metastable formulation, thermodynamic stability test were performed. Selected formulations were centrifuged at 3500 rpm for 30 min. Those formulations that did not show any phase separations were taken for heating and cooling cycle. Six cycles between refrigerator temperature of 4°C and 45°C for 48 h were done. The formulations that were stable at these temperatures were subjected to the freeze-thaw cycle test. Three freeze-thaw cycles were done for the formulations between -21°C and +25 °C. The formulations that survived dispersion stability tests were selected for further studies and the compositions of these formulations are given in Table 1.

Table 1. Composition of selected formulations

Formulation code	S_{mix} ratio	Oil: S_{mix} ratio	% w/w of components in nanoemulsion formulation		
			Oil	S_{mix}	Water
A1	1:1	1:9	5.18	48.64	46.18
A2	1:1	2:8	9.83	40.95	49.17
A3	1:1	3:7	12.09	29.45	58.46
B1	2:1	1:9	4.00	38.74	57.32
C1	1:2	1:9	6.40	57.70	35.62

Characterization of nanoemulsion

Droplet size distribution of the nanoemulsion was determined by photon correlation spectroscopy (PCS), using a Delsa Nano-C (Beckman Coulter Instruments, USA). Light scattering was monitored at 25°C at a scattering angle of 90°. Viscosity of the sample was measured using a Brookfield DV-II + Pro programmed cone and plate rheometer (Brookfield Engineering Laboratories Middlebore, USA) fitted with a cP-18 cone spindle and operating software Rheocale 32 software. The jacketed sample cup was connected to a circulating water bath operating at 25°C. Refractive index of nanoemulsion formulation was determined using an Abbes type refractrometer (Precision Standard Testing Equipment Corporation, India).

Table 2. Droplet size, viscosity, refractive index result of nanoemulsion formulations

Code	Droplet Size (nm)	Viscosity (cP)*	Refractive index
A1	21.0	40.17 ± 1.00	1.390 ± 0.0054
A2	117.9	238.66 ± 1.15	1.388 ± 0.0055
A3	105.5	245.33 ± 1.52	1.382 ± 0.0051
B1	23.3	41.66 ± 1.52	1.386 ± 0.0055
C1	58.1	40.66 ± 0.52	1.377 ± 0.0015

*Mean ± SD, $n = 3$.

Stability studies

From droplet size, viscosity, refractive index study result revealed that optimized A1 formulation were taken to perform stability studies 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 RI were determined using methods described above during storage. Accelerated stability studies were also performed on optimized tamoxifen citrate nanoemulsion. Three batches of formulations were taken in glass vials and were kept at accelerated temperature of 30°C, 40°C, 50°C and 60°C at ambient humidity. The samples were withdrawn at regular intervals of 0, 1, 2 and 3 months and were analyzed for drug content by UV Spectrophotometer at 272 nm.

The amount of drug degraded and the amount remaining at each time interval was calculated. Order of degradation was determined by the graphical method. 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 (K_{25}) was determined by extrapolating the value of 25°C from Arrhenius plot. The shelf life ($T_{0.9}$) for each formulation was determined by using the formula: $T_{0.9} = 0.1052/K_{25}$

Results and Discussion

Stability of a dosage form refers to the chemical and physical integrity of the dosage unit and when appropriate, the ability of the dosage unit to maintain protection against microbiological contamination. An ideal emulsion formulation must be thoroughly characterized physically, chemically and microbiologically at the start of study and throughout the intended shelf life period (Floyd 1999). 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 temperatures of 4°C 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 at both temperatures (Table 3).

Table 3. Droplet size, viscosity and refractive index of optimized A1 nanoemulsion formulation during storage

Time (Months)	Temperature (°C)	Droplets Size (nm)	Viscosity (cps)	Refractive index
0	4.0 ± 0.5	21.0	40.17 ± 1.00	1.390 ± 0.0054
1	4.0 ± 0.5	21.0	41.25 ± 1.24	1.392 ± 0.0051
2	4.0 ± 0.5	22.0	41.80 ± 1.10	1.393 ± 0.0048
3	4.0 ± 0.5	22.0	42.00 ± 1.50	1.393 ± 0.0052
0	25 ± 0.5	21.0	40.17 ± 1.00	1.390 ± 0.0054
1	25 ± 0.5	22.0	42.18 ± 1.50	1.394 ± 0.0041
2	25 ± 0.5	23.0	43.20 ± 1.30	1.396 ± 0.0038
3	25 ± 0.5	23.0	43.80 ± 1.70	1.395 ± 0.0035

These results indicated that optimized formulation is stable and suitable for transdermal delivery of tamoxifen citrate. 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 UV Spectrophotometer at 272 nm. The amount of tamoxifen citrate degraded and remaining in nanoemulsion formulation was determined at each time interval. The degradation of tamoxifen citrate was very slow at each temperature that could be stability of tamoxifen citrate in the form of nanoemulsion.

Observation is given in Table 4. Order of degradation was determined by graphical method at each temperature. The order of degradation was found to be first order (Fig. 1). The correlation coefficient of first order degradation was significant as compared to correlation coefficient of zero order degradation at each temperature as shown in Fig. 1 and 2. Therefore for first order degradation Log % of drug remaining was plotted against time and degradation rate constant (K) was calculated from the slope of the curve at each temperature as shown in table 4. The degradation rate constant was calculated by the formula: Slope = -K/2.303

Table 4. Degradation of optimized A1 nanoemulsion formulation

Time (Months)	Temp (°C)	Initial concentration (mg)	Concentration degradation (mg)	% remained	Log % remained
0	30 ± 0.5	2.66	0.00	100.00	2
1	30 ± 0.5	2.65	0.01	99.62	1.998347
2	30 ± 0.5	2.65	0.01	99.62	1.998347
3	30 ± 0.5	2.64	0.02	99.24	1.996687
0	40 ± 0.5	2.66	0.00	100.00	2
1	40 ± 0.5	2.64	0.02	99.24	1.996687
2	40 ± 0.5	2.62	0.04	98.49	1.993392
3	40 ± 0.5	2.60	0.06	97.63	1.988381
0	50 ± 0.5	2.66	0.00	100.00	2
1	50 ± 0.5	2.63	0.03	98.87	1.995065
2	50 ± 0.5	2.60	0.06	97.75	1.990117
3	50 ± 0.5	2.59	0.07	97.36	1.988381
0	60 ± 0.5	2.66	0.00	100.00	2
1	60 ± 0.5	2.63	0.03	98.87	1.995065
2	60 ± 0.5	2.56	0.10	97.36	1.988381
3	60 ± 0.5	2.54	0.12	95.49	1.979958

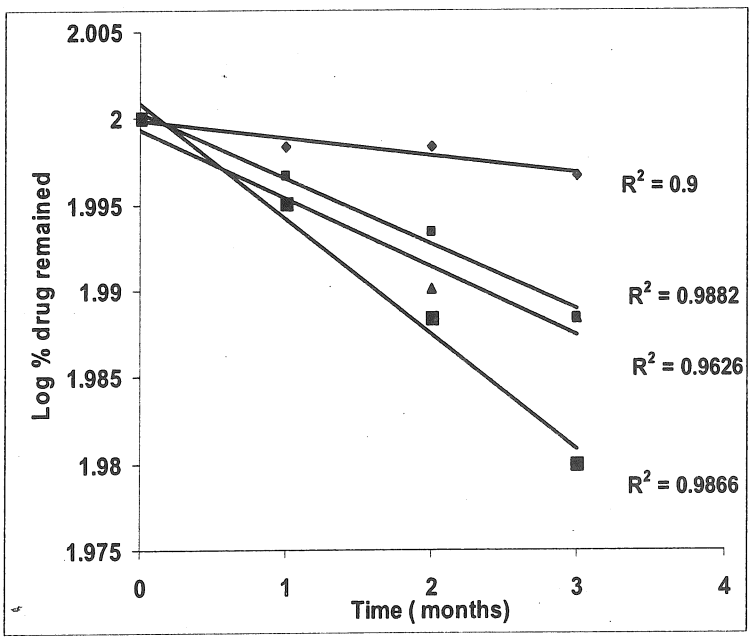

Figure 1. First order degradation kinetics of tamoxifen citrate from nanoemulsion formulation at different temperatures

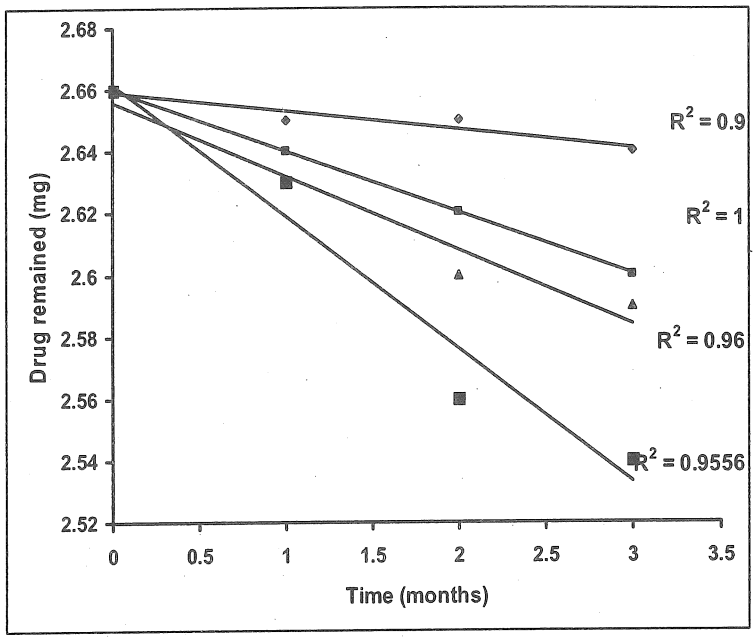

Figure 2. Zero order degradation kinetics of tamoxifen citrate from nanoemulsion formulation at different temperatures

The effect of temperature on the degradation was studied by plotting (Table 5) Log versus 1/T (Fig. 3). The value of K at 25° C (K 25) was obtained by extrapolation of the plot and shelf life was than calculated by substituting K_{25} in the following calculation.

$$T_{0.9} = 0.1052/K_{25}$$

Where $T_{0.9}$ is the time required for 10% drug degradation and is referred to as shelf life. The shelf life of optimized nanoemulsion formulation was found to be 2.05 years.

Table 5. Observation table for calculation of shelf life of nanoemulsion formulation

Temp (°C)	Slope	K x 10^{-3} (months^{-1})	Log K	Absolute Temperature	1/T x 10^{3}
30	-0.0010	2.303 ·	-2.637	303.00	3.300330
40	-0.0038	8.750	-2.057	313.00	3.194488
50	-0.0040	9.212	-2.035	323.00	3.095975
60	-0.0067	1.54	-2.812	333.00	3.003003
25	-------	4.265	-2.370	298.00	3.355704

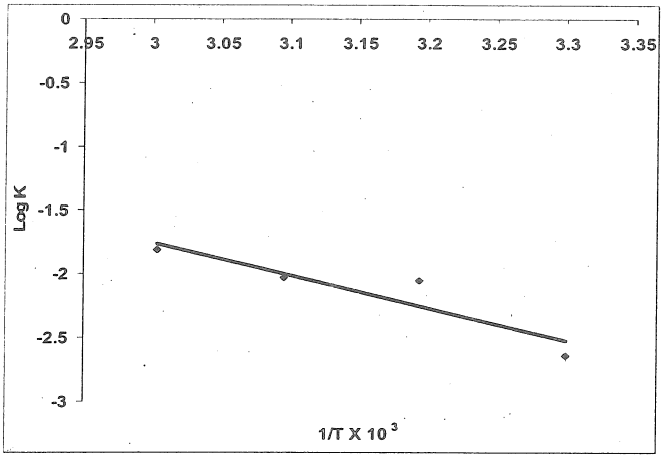

Figure 3. Arrhenius plot between Log K and 1/T for nanoemulsion formulation

Conclusion

The droplet size, viscosity and refractive index of optimized nanoemulsion formulation were not significantly changed during 3 months of storage. Therefore it was concluded that prepared nanoemulsion was physically stable. The degradation of tamoxifen citrate after 3 months of storage was very low in the nanoemulsion formulation. The shelf life of nanoemulsion formulation was found to be 2.05 years at room temperature. Overall these results revealed that stability of tamoxifen citrate can be enhanced in nanoemulsion formulation using cremophor RH 40 as surfactant.

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