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Research Article

COMPARISON OF STABILITY AND SHELF LIFE OF INJECTABLE *IN SITU* GELLING IMPLANT SYSTEMS OF DEFLAZACORT

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ABSTRACT

The objective of the research work was to evaluate and compare the stability and shelf life of *in situ* implant systems of anti-inflammatory drug, Deflazacort. *In situ* forming implants had gained wide attention as a formulation approach for those drugs intended to be used in chronic conditions where drug usage extended for weeks or months. Many limitations are reported to be associated with these delivery systems of which poor stability and short shelf life was major and need to be addressed. In this research work, i*n situ* implant formations of Deflazacort was designed using combination of poloxamer 188 and poloxamer 407 as thermo responsive polymers in one approach while in the other PLGA copolymer was used. Evaluation of the formulations were done based on gelation temperature, gel melting temperature, gelling time, gel duration and % entrapment efficiency. Both formulations showed absence of any significant changes in formulation characteristics during stability study period, indicating high stability. Shelf life studies concluded that, *in situ* implant systems based on Poloxamer 188 and 407 exhibited a shelf life of1.86 years, while PLGA based formulations, had a shelf life of 2.083years.

INTRODUCTION

Parenteral injectable in situ forming implants (ISFI)

Recent research studies has explored the potential of parenteral *in situ* forming implants (ISFI) as an attractive alternative compared the existing preformed implants.[1] ISFI which can be administered through parenteral route as injections avoid the use of invasive surgical procedure which are associated with administration conventional preformed implant devices. Also their manufacturing methods are simple, reproducible and feasible for scale up. They are available as solutions and are injected through subcutaneous or intramuscular route. Upon reaching inside the body, under the influence of a trigger response they transform to a gel or solid implant. [2]Various triggers are reported to be adopted to stimulate this transformation, which includes temperature, pH, cross linking, ion exchange etc.[3]Though the principle of ISFI looks attractive, there are limitations that need to be attended. Among the various limitations, burst release, toxicity issues and stability tops the list.[4, 5] Limited researches has been reported to overcome these limitations. Hence in this article, a study on stability and comparison of shelf life of *in situ* forming implant of Deflazacort, designed by two different approaches will be undertaken. Deflazacort, a novel anti-inflammatory drug is aglucocorticoid used clinically as an anti-inflammatory and immunosuppressant. It is indicated in treatment of rheumatoid, juvenile arthritis, ulcerative colitis, muscular dystrophy andnephrotic syndrome.[5]Considering the above facts, this study was undertaken with an objective to formulate and evaluate an *insitu* parenteral implantable controlled drug delivery system of Deflazacort, which could exhibit high stability and satisfactory shelf life.

MATERIALS AND METHODS

Materials

Deflazacort was obtained as a gift sample from German remedies, Goa. Poloxamer 188 and 407 were purchased from Fizmerk India chemicals, Hapur (U.P).

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Methods

Formulation development of Deflazacort in situ implant based on Poloxamer 188 and 407

Formulation FT-13 [Deflazacort *in situ* implant based on Poloxamer 188 and 407], was designed as per the composition given in Table No: 1Add required quantity of polymer slowly into cold water maintained at a temp of 4-8ºC. Stir this solution using a magnetic stirrer at the same temperature. Once mixing is completed, the container is sealed and kept overnight in the refrigerator at a temperature of 4-8 ºC. This yields a clear solution after 12 hours. Deflazacort was dissolved in DMSO with stirring. The drug solution is then added to polymer solution with stirring at 2000 rpm for 30 minutes. Prepared solution was packed and sealed into containers.[6]

Table No. 1 Formulation of Deflazacort *in situ* implant systems based on Poloxamer

Formulation Code	Ingredients (% w/v)				
FT-13	Deflazacort	Poloxamer 188	Poloxamer 407	HPMC K4M	Purified water
	10.00	10.00	17.00	1.00	62.00

Formulation development of Deflazacort in situ implant based on PLGA copolymers

Preparation of insitu formulations of Deflazacort

In the preparation of FPH-2 [Deflazacort *in situ* implant based on PLGApolymer], accurately weighed PLGA polymer was added into the vortex created by stirring of DMSO solution with a mechanical stirrer. Stirring was done to form a uniform mixture. Accurately weighed quantity of Deflazacort was added to the above polymer solution. The resultant solution was stirred at 2000 rpm for 30 minutes to form a uniform mixture. Volume was made up with vehicle.

Table No. 2 Formulation of Deflazacort *in-situ* implant formulation based on PLGA

Formulation code	Ingredients (% w/v)			
FPH-2	Drug	PLGA 85:15	HPMC K4M	DMSO
	10.00	30.00	2.00	58.00

Stability Studies[7]

Development of any pharmaceutical product is not complete without proper stability analysis. The stability studies were carried out to assess physical and chemical stability of the product. Stability refers to chemical and physical integrity of dosage unit to maintain protection against physical, chemical and microbial contamination. Stability testing was performed as per ICH guidelines for optimized formulation at 40± 2°C and 75 ± 5 % RH, for a period one month. On completion of stability period the samples were withdrawn and estimated for physical appearance, pH, gelling time, gel duration, gelation temperature, mucoadhesive strength, viscosity, syringeability time, isotonicity, drug content and drug diffusion.

Determination of shelf life[8]

Determination of shelf life is a vital phase during formulation and development of any dosage form. Shelf life determination predicts the expiry period of formulation and also establishes the ideal storage conditions which could provide improved stability and extended shelf life. This study is based on the principle of accelerated stability studies making use of Arrhenius equation and its associated graphical plots. The formulation subjected for the study was FP13 and objective was to establish its shelf life at 25 ± 2°C / 60 ± 5% RH. For this, the optimized FP13 formulation were kept at 30 ± 2°C / 60 ± 5% RH, 40 ± 2°C / 60 ± 5% RH and 50 ± 2°C / 60 ± 5% RH for three months. Samples were withdrawn and analyzed for residual drug content at specified time intervals of 0, 30, 60, and 90 days. Samples of zero time were used as controls. From the determined drug content, the order of the degradation reaction should be first determined. Once the order of reaction is determined, degradation rate constant [K] should be determined. For this a plot of drug concentration against time (in months) need to be plotted. The degradation rate constant [K] was determined from the slope of the linear line. Thus degradation rate constant [K] at each experimental temperature [K_{30}, K_{40}, K_{50}] will be calculated. Arrhenius plot was then constructed between the determined log K values at various elevated temperatures against the reciprocal of absolute temperature (1/T). From the Arrhenius plot the degradation rate constant at 25°C (K25) can be determined for by graphical extrapolation or using linear equation. The shelf life (T_{90}) for formulation was determined by substituting the value of K_{25} in the respective equation based on the order of reaction.

Drug Content Estimation[9]

Test formulation containing 100 mg of Deflazacort was taken. 1ml of this test solution is then diluted suitably with ethanol. Absorbance of the diluted test solution was measured by using UV Visible spectrometer at 246nm.

Table No.3 Stability studies data of FTH-2

Physical Appearance			
Initial		**After stability**	
Clear		Clear	
pH			
Initial		**After stability**	
7.2 ± 0.03		7.1 ± 0.2	
Gelling Time			
Initial		**After stability**	
5.4±2 min		5.6±1 min	
Gel duration			
Initial		**After stability**	
172 ± 2.1.hr		171 ± 1.6 hr	
Gelation temperature			
Initial		**After stability**	
34.6 ± 0.0.26ºC		34.8 ± 0.15ºC	
Gel melting temperature			
Initial		**After stability**	
52.1 ± 0.25ºC		51.9 ± 0.14ºC	
Viscosity			
Initial		**After stability**	
At 25±0.5ºC	**At 37±0.5ºC**	**At 25±0.5ºC**	**At 37±0.5ºC**
2543 ±2.3 cps	58722 ± 2.5 cps	2581 ±4.1 cps	59831 ± 2.2cps
Syringeability Time			
Initial		**After stability**	
6.5 ± 0.3 4.2±0.2 sec		6.7 ± 0.4 4.0±12 sec	
Drug Content			
Initial		**After stability**	
99.7 ± 0.2 %		98.8. %	

RESULTS

Stability Studies

Stability data of Deflazacort in situ implant based on Poloxamer 188 and 407

Stability studies were conducted to determine the shelf life and storage condition of a product. In this investigation FTH-2 was subjected to accelerated stability studies for a period of 1 month. Accelerated stability studies were performed in accordance with ICH guidelines with certain modifications. The studies were carried out to verify the changes in physical appearance, pH, gelling time, gel duration, gelation temperature, gel melting temperature, viscosity, syringeability time, drug content and drug diffusion at (40±2^{0}C) for 3 month. The results are reported in Table No.3&4 and Fig No.1

Table No. 4 In-vitro Diffusion profile of FTH-2 on stability

Time(hr)	Cumulative Percentage drug release (%)	
	Before stability	After stability
0	0	0
6	06.39 ± 0.29	0.7.25±0.46
12	10.09 ± 0.30	11.26±0.24
24	14.57 ± 0.14	16.45±0.34
48	27.45 ± 0.23	29.56±0.65
72	41.47 ± 0.15	42.38±0.47
96	56.29 ± 0.34	57.38±0.28
120	70.74 ± 0.47	72.45±0.37
144	84.23 ± 0.14	85.28±0.35
168	98.28 ± 0.24	98.12±0.23
192	97.65 ± 0.53	97.45±0.25

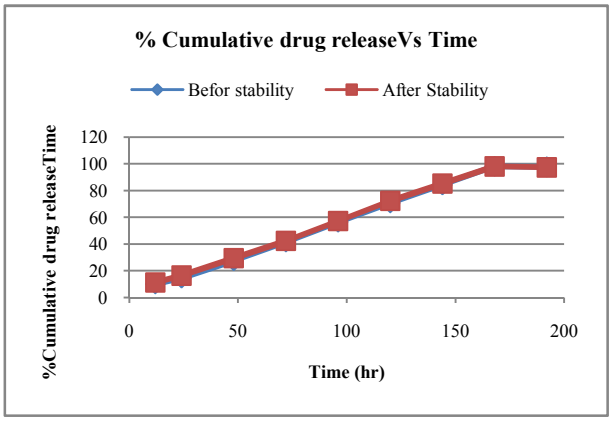


Fig No. 1 In-vitro Diffusion profile of FTH-2

Stability data of Deflazacort in situ implant based on PLGA copolymer

Stability studies were used to determine the shelf life and storage condition of a product. In this investigation FPH-2 were subjected to accelerated stability studies for a period of 1 month. Accelerated stability studies were performed in accordance with ICH guidelines with certain modifications. The studies were carried out to verify the changes in physical characteristics such as physical appearance, pH, gelling time, gel duration, gelation temperature, gel melting temperature, viscosity, syringeability time, drug content and drug diffusion at (40±2^{0}C) for 3 months. The results are reported in Table No: 5&6 and Fig No.2

Table no.5 Stability studies data of FPH-2

Physical Appearance			
Initial		**Stability(after a period of 3 months)**	
Clear		Clear	
pH			
Initial		**After stability**	
7.2 ± 0.02		7.1 ±0.2	
Gelling Time			
Initial		**After stability**	
5.22 ± 0.02min		5.4 ± 1 min	
Gel duration			
Initial		**After stability**	
30 ±1		29 ± 2	
Viscosity			
Initial		**After stability**	
Room Temp TttgghdtetTemperature Temtttemperature	**At 37±0.5ºC**	**Room Temp**	**At 37±0.5ºC**
6932 ± 2.9 cps	32563 ±5.2cps	6900 ±3.1cps	31899±2.2cps
Syringeability Time			
Initial		**After stability**	
6.8 seconds 4.2±0.2 sec		6.3 seconds 4.0±12 sec	
Drug Content			
Initial		**After stability**	
98.58 ± 0.64 %		97.68 ± 0.54 %	

Table No. 6 In-vitro Diffusion profile of FPH-2 upon stability studies

Time (days)	Cumulative % drug release	
	Before stability	After stability
0	0	0
1	4.15 ± 0.46	4.25 ± 0.28
2	7.25 ± 0.74	8.01 ± 0.66
4	13.48 ± 0.25	12.57±0.38
6	21.76 ± 0.64	23.52±0.18
8	27.34 ± 0.74	28.03±0.63
12	42.11 ± 0.46	45.47±0.73
16	54.56 ± 0.26	58.27±0.95
20	69.42 ± 0.41	71.88±0.64
24	83.05 ± 0.36	85.32±0.75
28	97.23 ± 0.34	97.11±0.55

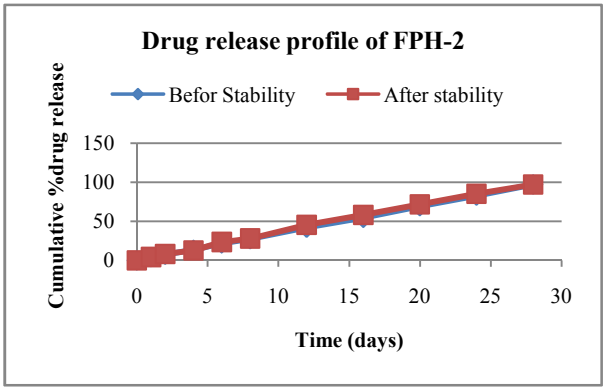


Figure No 2 Invitro Diffusion profile of FPH-2

The evaluation of formulations after stability charging showed there was no significant changes with respect to physical appearance, pH, gelling time, gel duration, gelation temperature, gel melting temperature, viscosity, syringeability time, drug content and In-vitro drug dissolution study with respect to results obtained before stability charging. Thus it was concluded that the formulations were found to possess stability compliance requirements as per ICH guidelines.

Determination of shelf life

Shelf life of Deflazacort in situ implant based on Poloxamer 188 and 407

The objective was to establish the shelf life of the FTH-2 formulation at the proposed storage temperature of 25 ± 2°C / 60 ± 5% RH. For this, the drug content of FTH-2 formulation was determined at elevated temperatures of 30^0C, 40^0C and 50^0C. From the plot of residual concentration of drug against time (months) the order of reaction was determined. The order of degradation was determined by fitting the drug degradation data into various pharmacokinetic models and determining the coefficient of correlation (R^2). A high coefficient of correlation (R^2) of 0.9995, 0.9820 and 0.9903 at temperatures of at 30 ± 2°C / 60 ± 5% RH, 40 ± 2°C / 60 ± 5% RH and 50 ± 2°C / 60 ± 5% RH indicated that the degradation followed first order kinetics. The same has been reported in Table No.7 & 8 and Fig No.3 & 4. Based on this, logarithm of percent drug remaining versus time (in months) were plotted. The degradation rate constant 'k' was determined from the slope of the linear line at each experimental temperature using the equation;

k = slope x 2.303

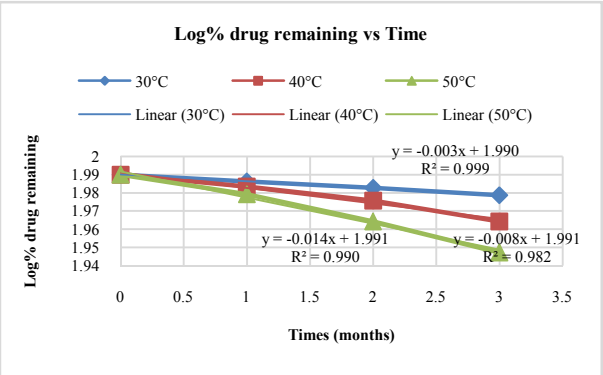
The plot of Log K against 1/T (Temperature in kelvin) gave a linear plot with straight line equation, y = -2878x + 7.4601 from which K_{25} was determined. Shelf life (t_{90})was determined from the equation

Shelf life = 0.105/ K_{25}

The results are given in table No 7& 8 and Fig.No. 3&4. A shelf life of 22.34 Months (1.86 Years) was calculated from the stability data.

Table No. 7 Determination of Degradation Rate Constant of FTH-2

Temp °C	Time	Concentration (mg/ml)	Concentration degraded (mg/ml)	%Drug remaining	Log % Drug remaining
	0	97.78	2.22	97.78	1.9900
	1	96.90	3.10	96.90	1.9863
30	2	96.10	3.90	96.10	1.9827
	3	95.22	4.78	95.22	1.9787
	0	97.78	2.22	97.78	1.9900
	1	96.27	3.73	96.27	1.9835
40	2	94.58	4.42	94.58	1.9758
	3	94.10	5.90	94.10	1.9643
	0	97.78	2.22	97.78	1.9900
	1	95.32	4.68	95.32	1.9792
50	2	92.11	7.89	92.11	1.9643
	3	88.56	11.44	88.56	1.9472



sFig No.3 Determination of Degradation Rate Constant of Fth-2

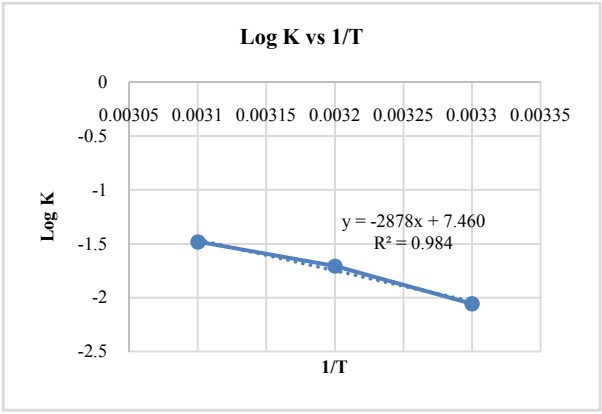


Fig No. 4 Log K Versus 1/T of FTH-2

Table No. 8 Determination of Shelf Life of AT 25°C

TEMP (°C)	Degradation Reaction Rate Constant (K)	LOG K	Absolute Temp (T)	1/T	Shelf Life (t_{90})
25	0.0047	-2.3251	298	0.0034	22.34
30	0.0086	-2.0576	303	0.0033	Months
40	0.0196	-1.7082	313	0.0032	(1.86
50	0.0329	-1.4824	323	0.0031	Years)

Determination of shelf life Deflazacort in situ implant based on PLGA copolymer

The objective was to establish the shelf life of the FPH-2formulation at the proposed storage temperature of 25 ± 2°C / 60 ± 5% RH. For this, the drug content of FPH-2formulation was determined at elevated temperatures of 30^0C, 40^0C and 50^0C. From the plot of residual concentration of drug against time (months) the order of reaction was determined. The order of degradation was determined by fitting the drug degradation data into various pharmacokinetic models and determining the coefficient of correlation (R^2). A high coefficient of correlation (R^2) of 0.9963, 0.988 and 0.9633 at temperatures of at 30 ± 2°C / 60 ± 5% RH, 40 ± 2°C / 60 ± 5% RH and 50 ± 2°C / 60 ± 5% RH indicated that the degradation followed first order kinetics. The same has been reported in Table No.9 & 10 and Fig No.5 & 6. Based on this, logarithm of percent drug remaining versus time (in months) were plotted. The degradation rate constant 'k' was determined from the slope of the linear line at each experimental temperature using the equation;

k = slope x 2.303

The plot of Log K against 1/T (Temperature in kelvin) gave a linear plot with straight line equation, y = -3054.5x + 8.0083from which K_{25} was determined. Shelf life (t_{90}) was determined from the equation

Shelf life = 0.105/ K_{25}

The results are given in table No.9&10 and Fig.No: 5 & 6. A shelf life of 22.34 Months (1.86 Years) was calculated from the stability data.

Table No. 9 Determination of Degradation Rate Constant of FPH-2

Temp °C	Time	Concentration (mg/ml)	Concentration degraded (mg/ml)	% Drug remaining	Log % Drug remaining
30	0	98.88	1.12	98.88	1.9951
	1	97.90	2.10	97.90	1.9907
	2	97.10	2.90	97.10	1.9872
	3	96.01	3.99	96.01	1.9823
40	0	98.88	1.12	98.88	1.9951
	1	97.15	2.85	97.15	1.9874
	2	95.98	4.02	95.98	1.9822
	3	95.10	3.90	95.10	1.9782
50	0	98.88	1.12	98.88	1.9951
	1	94.92	5.08	94.92	1.9774
	2	92.89	7.11	92.89	1.9680
	3	87.26	12.74	87.26	1.9408

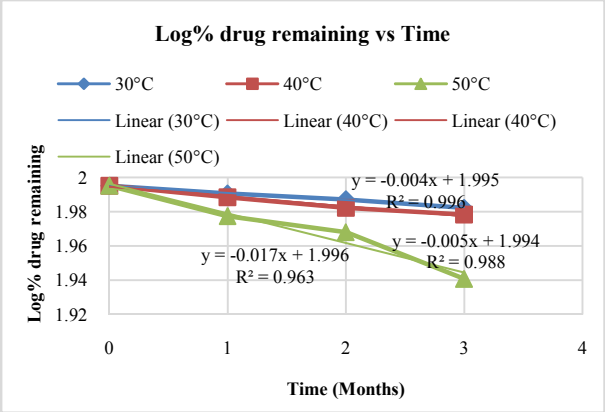


Fig No. 5 Determination of Degradation Rate Constant of FPH-2

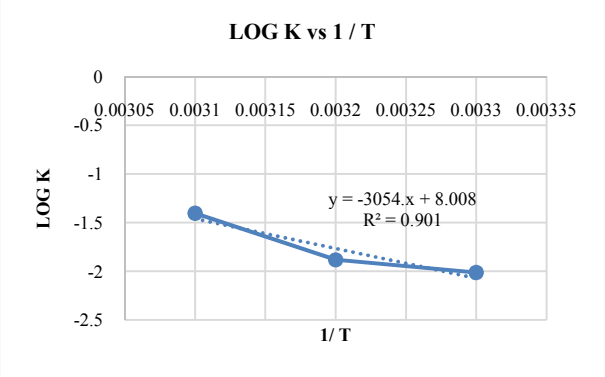


Fig No. 6 Log K Versus 1/T OF FPH-2

Table No. 10 Determination of Shelf Life of AT 25°C

Temp (°C)	Degradation Reaction Rate Constant (K)	Log K	Absolute Temp (T)	1/T	Shelf Life (t_{90})
25	0.0042	-2.377	298	0.0034	25.0
30	0.0097	-2.3013	303	0.0033	Months
40	0.0131	-1.8827	313	0.0032	(2.083
50	0.0396	-1.4023	323	0.0031	Years)

Table No. 11 Comparison of Stability and Shelf Life

Formulation	Stability	Shelf Life (T_{90})
FTH-2	Stable	22.34 Months (1.86 Years)
FPH-2	Stable	25.0 Months (2.083 Years)

CONCLUSION

The accelerated stability study concluded that both poloxamer and PLGA based *in situ* implant systems of anti-inflammatory drug, Deflazacort remained stable without any significant changes in formulation characteristics. The shelf life of these formulations as provided in Table No. 11, were also found to be satisfactory with poloxamer based *in situ* implant systems exhibiting 1.86 years, whereas PLGA based *in situ* implants showing 2.083 years. Thus we can propose the intended storage condition to be 25 ± 2°C / 60 ± 5% RH, the room temperature. The study proved the potential of polymer based *in situ* implant systems of anti-inflammatory drug, Deflazacort to provide a stable formulation with satisfactory shelf life. Further scale up studies and clinical studies need to be done to establish the reproducibility and safety of these formulations.

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Conflicts of Interest

The authors declares that there is no conflict of interest regarding the publication of this article.

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