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FORMULATION AND EVALUATION OF PEMETREXED DISODIUM LOADED PLGA MICROSPHERES

Vanga Harish*[1], Budidi Krishna Geetha[1], Lohithasu D.[2], Prashanth Kumar Killi[3] and M. Mohan Varma[1]

[1]Shri Vishnu College of Pharmacy, Bhimavaram, W.G.Dt.

[2]AU College of Pharmaceutical Sciences, Andhra University, Visakhapatnam.

[3]Sree Datta Institute of Pharmacy, Ibrahimpatnam, Hyderabad.

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***Corresponding Author**
Vanga Harish
Shri Vishnu College of Pharmacy, Bhimavaram, W.G.Dt.

ABSTRACT

Objective: The objective of the present work was to formulate and evaluate controlled release Pemetrexed disodium microspheres for subcutaneous administration for treating pleural mesothelioma and non-small cell lung cancer effectively and also to improve patient compliance with fewer side effects. Pemetrexed is chemically similar to folic acid (L-Glutamic acid) and is in the class of chemotherapy drugs called folate antimetabolites. It works by inhibiting three enzymes used in purine and pyrimidine synthesis - thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). By inhibiting the formation of precursor purine and pyrimidine nucleotides, pemetrexed prevents the formation of DNA and RNA, which are required for the growth and survival of both normal cells and cancer cells. ***Method:*** Different formulations were prepared by following solvent evaporation technique (double emulsion) using biodegradable poly(Lactide-*co*-Glycolide) acid and evaluated for percentage yield, entrapment efficiency, surface morphology (SEM), particle size analysis, *in-vitro* drug release and stability studies. ***Results:*** The prepared microspheres were white, free flowing and spherical in shape. The mean Particle size of the microspheres was found in the range of 26 to 206µm. The drug-loaded microspheres showed 87% of entrapment and release was extended up to 30 days releasing 95.6% of the total drug from the microspheres. The infrared spectra showed stable character of pemetrexed in the drug-loaded microspheres and revealed the absence of drug-polymer interactions. Scanning electron microscopy study revealed that the microspheres were spherical and porous in nature.

Conclusion: The pemetrexed is uniformly distributed within the microspheres which are made of a biodegradable D, L-lactic and glycolic acids co-polymer. The optimized formulations of Pemetrexed disodium microspheres with controlled release were attempted for a release upto atleast one month.

KEYWORDS: Bioavailability, Controlled release, Microspheres, Pemetrexed disodium, PLGA polymer, SEM, Subcutaneous.

INTRODUCTION

Pemetrexed is chemically similar to folic acid (L-Glutamic acid) and is in the class of chemotherapy drugs called folate antimetabolites. It works by inhibiting three enzymes used in purine and pyrimidine synthesis - thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). By inhibiting the formation of precursor purine and pyrimidine nucleotides, pemetrexed prevents the formation of DNA and RNA, which are required for the growth and survival of both normal cells and cancer cells. The FDA has approved pemetrexed for treatment of malignant Pleural Mesothelioma, a type of tumor of the lining of the lung.

Table 1: Marketed formulations of pemetrexed disodium.

S.No	Proprietary name	Company	Pharmaceutical Formulations
1	Alimta	Eli-Lilly	It s supplied as a sterile lyophilized powder for intravenous infusion available in single-dose vials. Each 100 mg or 500 mg vial contains Pemetrexed disodium equivalent to 100 mg Pemetrexed and 106 mg mannitol or 500 mg Pemetrexed and 500 mg mannitol respectively.
2	Pemgem	Dr.Reddey's	
3	Pemnat	Natco pharma	
4	Prex	Celon laboratories	
5	Pemetrexed	Intas pharma	

The aim of the present research was to formulate and evaluate pemetrexed disodium microspheres for subcutaneous administration for period of one month controlled release delivery system. Biodegradable microspheres were shown to improve the bioavailability of peptides by protecting them from physical degradation and proteolysis in body fluids. Poly (D,L-lactide) (PLA) and poly (D,L-lactide-*co*-glycolide) (PLGA) are the most widely used and well-characterized materials for the preparation of biodegradable microspheres.

MATERIALS AND METHODS

Materials

Pemetrexed disodium was purchased from Salius Pharma (Mumbai). Poly (D, L-Lactic-*co*-glycolic acid) 50:50 (PLGA 50:50) Resomer®RG 504 was supplied by Sigma Aldrich. Poly Vinyl Alcohol (PVA) (MW 22000, 88% hydrolyzed) was supplied by S.D fine chemicals, Mumbai. Dichloromethane and Ethyl acetate were supplied by Qualigens.

Preparation of Pemetrexed disodium microspheres

Pemetrexed disodium-loaded PLGA microspheres were prepared by a double emulsion-solvent evaporation technique. Briefly, 500 mg PLGA 50:50 was dissolved in 2 mL dichloromethane (oil phase). An aqueous solution containing 100 mg of pemetrexed disodium in 0.2 ml of water was prepared separately (inner aqueous phase or W_1). The first aqueous (W_1) phase was emulsified into the oil phase (containing PLGA), using a high-speed homogenizer (Ika® T25 Digital Ultra Turrax) operating around 10000 rpm for about 1 minute at 2-8ºC to form water in oil(W_1/O) primary emulsion. This primary emulsion was added in to 50 ml of external aqueous phase containing 1% PVA solution to form secondary emulsion(W_1/O/W_2) at homogenizer speed around 8000 rpm for 3 minutes and then stirred at 1000 rpm for 1 hour at 2-8 ^{0}C, then next 2 hrs at room temperature to permit evaporation of dichlomethane. The wet microspheres obtained were collected by centrifugation followed by filtration and Lyophillization.

Different formulation variables like amount of PLGA (F1, F2 & F3), volume of Ethyl Acetate (F4 & F5), Volume of Dichloromethane(F6 & F7) were carried out as below mentioned Table 2.

Table 2: Formulation variable of Pemetrexed disodium microspheres

COMPOSITION		FORMULATIONS						
		F1	**F2**	**F3**	**F4**	**F5**	**F6**	**F7**
Drug (mg)		100	100	100	100	100	100	100
WFI (ml)		0.2	0.2	0.2	0.2	0.2	0.2	0.2
PLGA 50:50 (mg)		**1000**	**700**	**300**	500	500	500	500
DCM (ml)		2	2	2	-	-	**2**	**4**
PVA 1% (ml)		50	50	50	50	50	50	50
Ethyl acetate		-	-	-	**4**	**2**	-	-
Temperature (ºC)		2-8	2-8	2-8	2-8	2-8	2-8	2-8
Homogenization speed (rpm)	1° (5min)	10,000	10,000	10,000	10,000	10,000	10,000	10,000
	2° (3min)	8000	8000	8000	8000	8000	8000	8000

Process variables like secondary homogenization speed (F8, F9 & F10) and primary homogenization speed (F11 & F12) were carried out as below mentioned Table 3.

Table 3: Process variable of pemetrexed disodium microspheres.

COMPOSITION		FORMULATIONS				
		F8	**F9**	**F10**	**F11**	**F12**
Drug (mg)		100	100	100	100	100
WFI (ml)		0.2	0.2	0.2	0.2	0.2
PLGA 50:50 (mg)		500	500	500	500	500
DCM (ml)		2	2	2	2	2
PVA 1% (ml)		50	50	50	50	50
Ethyl acetate		-	-	-	-	-
Temperature (°C)		2-8	2-8	2-8	2-8	2-8
Homogenization speed (rpm)	1° (5min)	10,000	10,000	10,000	**12,000**	**14,000**
	2º (3min)	**4000**	**6000**	**8000**	8000	8000

EVALUATION OF PEMETREXED DISODIUM MICROSPHERES

Determination of percentage yield

Microspheres were weighed and the yield of microspheres was calculated using the formula:

Percentage yield = Practical yield (gm) / Theoretical yield × 100.

Determination of drug entrapment efficiency (EE):

The amount of drug entrapped was estimated by dispersing microspheres equivalent to 50 mg of Pemetrexed disodium in DCM and water in 3:1 ratio, under vigorous shaking for 1hr, the resultant solution was centrifuged. Both layers were separated. As the Pemetrexed disodium was soluble in water but not in DCM, the drug content in aqueous solution was analyzed by using HPLC at 254 nm with further dilutions against appropriate blank.

The amount of the drug entrapped in the microspheres was calculated using the formula.

% EE = Actual weight of drug in sample/ Theoretical weight of drug in sample x 100.

Particle size analysis

The mean diameter of microspheres was determined by laser diffractometer (Mastersizer X, Malvern Instrument, UK).

Microspheres were suspended in 0.3% aqueous solution of Tween 80 and sonicated for 15 s prior to particle size determination.

Scanning electron microscopy (SEM)

The morphology of microspheres was examined by scanning electron microscopy (Hitachi-S-3700N). The samples were prepared by sprinkling the microspheres on one side of adhesive stub. Then the microspheres were coated with gold (100A°) before microscopy.

***In-vitro* drug release**

The in-vitro drug release from the microspheres was carried out by using a regenerated cellulose membrane dialysis apparatus Float-A-lyzer. 2ml of microspheres suspension containing known amount of drug was placed in the Float-A-lyzer and this was placed in 50 ml of water, maintained at 37°C and stirred with the help of a magnetic stirrer. Aliquots (2ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh water to maintain constant volume. The samples were analyzed for drug content by HPLC at 254nm. Upon completion of one week, the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium.

Stability studies

To assess the physical and chemical stability of the microspheres, stability studies were conducted for 3 months under the storage conditions mentioned in ICH guidelines. The optimized formulation was placed in vials and stored at 25±2°C/ 60±5% RH. After 90 days the formulations were checked for physical appearance and drug content.

RESULTS AND DISCUSSIONS

Summarized results of formulation and process variable are given in below Table 4.

Table 4: Results of formulation variables of Pemetrexed disodium microspheres

Batches	Percentage Yield (%)	Entrapment Efficiency (%)	Rounded Mean Particle Size
F1	71.4	65	86
F2	70.5	77	83
F3	65.6	66	47
F4	50.6	57	56
F5	53.9	62	67
F6	72.8	84	91
F7	73.7	83	98
F8	72.2	82	74
F9	72.8	84	72
F10	**76.5**	**87**	**81**
F11	71.4	74	61
F12	60.2	67	53

Effect of polymer concentration

As the amount of polymer increased, the particle size of Pemetrexed disodium was found to increase and encapsulation efficiency was found to decrease. The polymer matrix might have saturated with pemetrexed disodium after a certain optimized concentration. Upon further increase in polymer, the free drug tends to escape from the polymer and therefore the encapsulation efficiency is found to decrease.

Effect of Organic Solvent

Two organic solvents namely ethyl acetate and dichloromethane were studied. When the polymer was dissolved in ethyl acetate, encapsulation efficiency was found to decrease because of its high boiling point (77.1^0C) which must have resulted in slow solvent removal rate. When the polymer was dissolved in dichloromethane (BP 39.6°C), high encapsulation efficiency was obtained indicating faster rate of solvent removal. Dichloromethane is also more soluble in water and its solubility allowed relatively fast mass-transfer between the dispersed and the continuous phase and led to fast precipitation of the polymer.

Effect of homogenization speed

When the secondary homogenization speed (rpm) was decreased in case of F8 & F9 formulations, the particle size was found to decrease. When this homogenization speed was increased to 8000 rpm in F10, particle size was found to be 81 micro meters which was optimized. In F11 and F12 formulations, as the primary homogenization speed was increased from 10,000 rpm to 14,000 rpm, further reduction in the particle size of microspheres was observed.

Effect of temperature

Low temperature (2-8ºC) was maintained to improve the formation of microspheres with high encapsulation efficiency.

From above results of all parameters, it was observed that the formulation F10 was given desired particle size, entrapment efficiency and initial burst release.

CHARACTERIZATION OF PEMETREXED LOADED MICROSPHERES

Formulation F10 was chosen for characterization of Pemetrexed loaded PLGA microspheres.

Mean particle size distribution

The mean diameter of microspheres was determined by laser diffractometer (Mastersizer X, Malvern Instrument, UK).

Microspheres were suspended in 0.3% aqueous solution of Tween 80 and sonicated for 15 s prior to particle size determination. The mean particle size of formulation F10 was shown below in Figure 1.

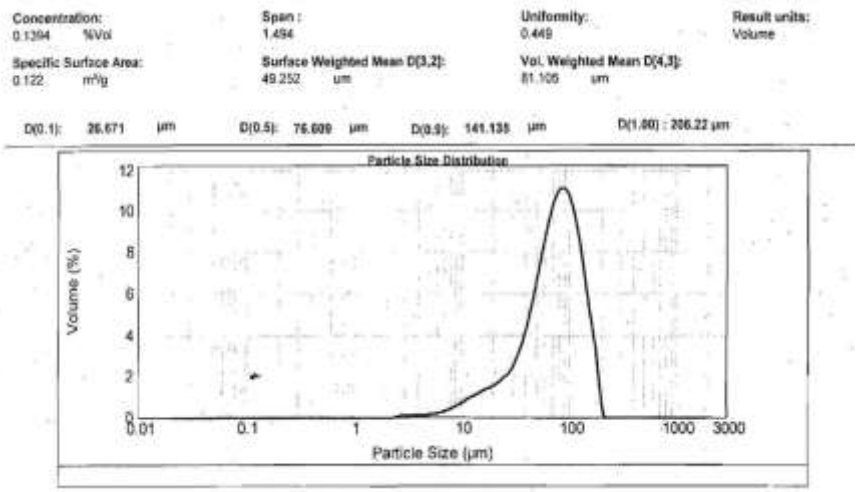

Figure 1: Mean Particle size of formulation F10

Scanning electron microscopy (SEM)

The morphology of microspheres was examined by scanning electron microscopy (Hitachi-S-3700N). Samples were mounted on metal stubs and sputter-coated with gold for 4 min prior to examination under. The SEM picture showed in Figure 2 that the shape of the microspheres was spherical and smooth surface with less porosity.

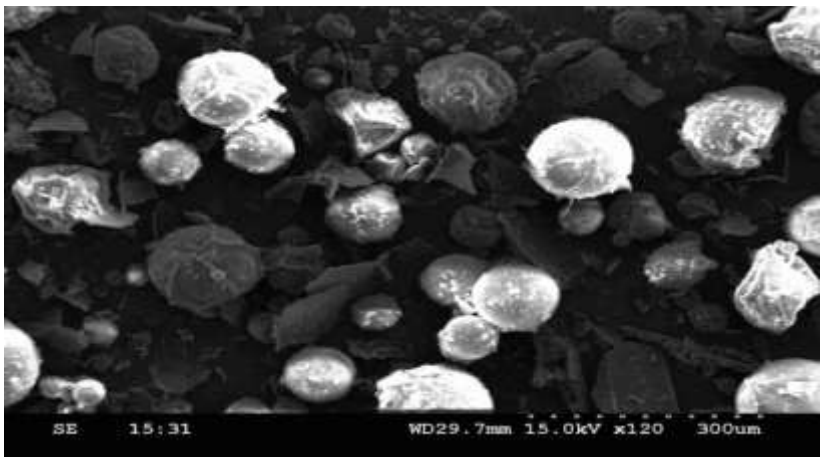

Figure 2: SEM picture of formulation F10.

***In-vitro* drug release**

The *in-vitro* drug release from the microspheres was carried out by using a regenerated cellulose membrane dialysis apparatus Float-A-lyzer. 2ml of microspheres suspension containing known amount of drug (50 mg microspheres) was placed in the Float-A-lyzer and this was placed in 50ml of water, maintained at 37°C and stirred with the help of a magnetic stirrer. Aliquots (2ml) of release medium were withdrawn at different time intervals and the sample was replaced with fresh water to maintain constant volume. The samples were analyzed for drug content by HPLC at 254nm. Upon completion of one week, the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium. Initial burst release means release of drug within 24 hrs and F10 has shown 18.2% IBR.

The cumulative percent release of F7, F9 & F10 formulations at various time intervals was calculated and was plotted against time in figure 4.

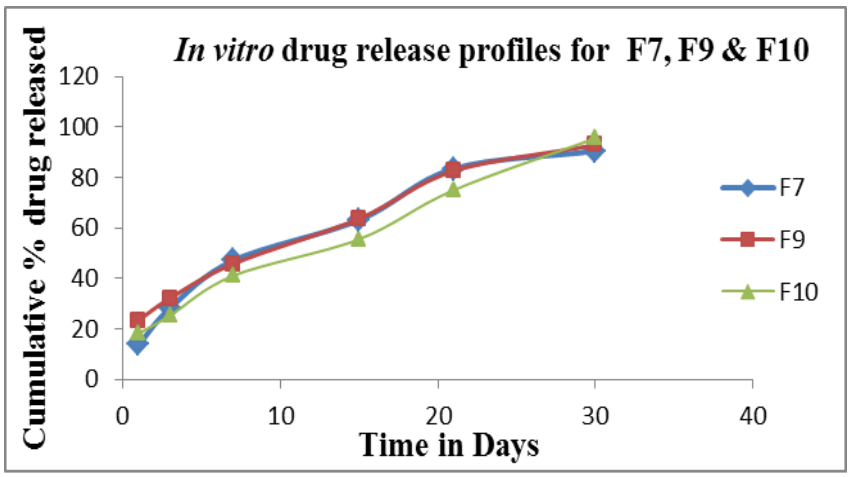

Figure 3: Comparison of *in-vitro* drug release profile of Pemetrexed disodium from the formulation F7, F9 and F10.

Release Kinetics

The release kinetics of F7, F9 & F10 formulations were studied. All formulations follow Higuchi release kinetics and follow Anomalous (non-Fickian) diffusion when it applied to the Korsmeyer-Peppa's Model for mechanism of drug release. F10 formulation has better kinetic results when compared to F7 and F9 formulations. The results are shown in Figure 5, 6, 7 & 8 and in Table 5.

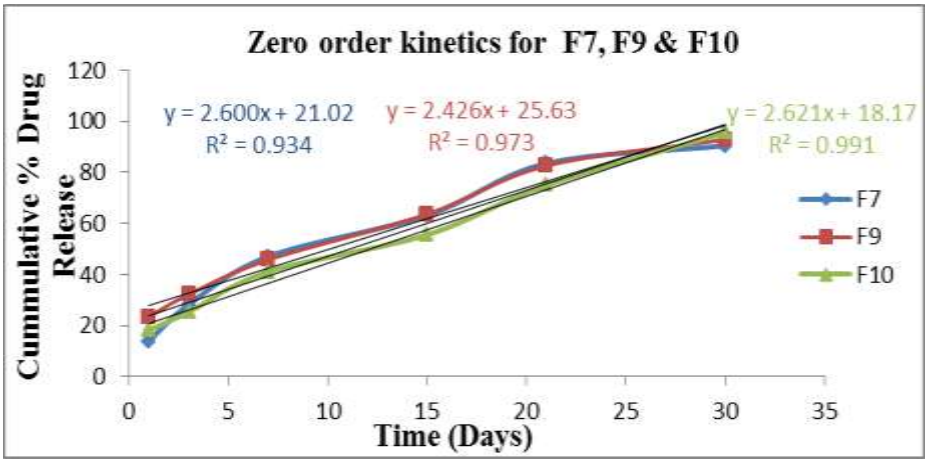

Figure 4: Comparison of Zero order Release profiles of optimized formulations F7, F9 and F10.

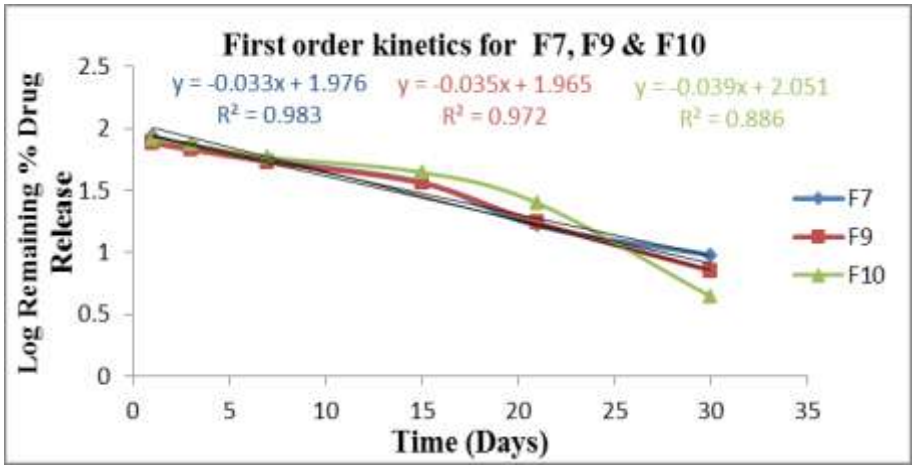

Figure 5: Comparison of First order Release profiles of optimized formulations F7, F9, F10.

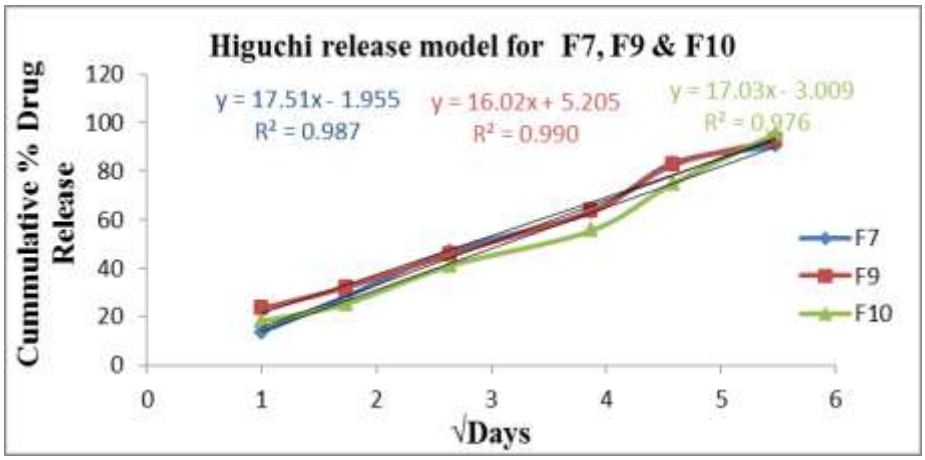

Figure 6: Comparison of Higuchi's order plot for optimized formulations F7, F9 and F10

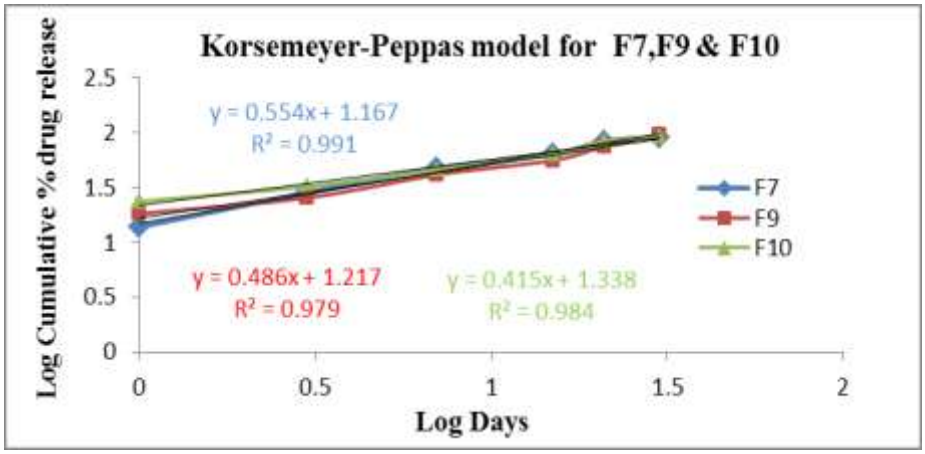

Figure 7: Comparison of Korsmeyer-Peppas model for optimized formulations F7, F9 and F10.

Table 5: Curve fitting data of drug release profiles for optimized formulations F7, F9 and F10.

Formulation Code	Zero Order (R^2)	First Order (R^2)	Higuchi (R^2)	Korsmeyer Peppas (N)
F7	0.934	0.983	0.987	0.554
F9	0.973	0.972	0.990	0.486
F10`	0.991	0.886	0.976	0.415

From the Table (5), R^2 value for formulation F10 was found to be more and was found to follow zero order kinetics, which states that the drug release is independent of concentration.

Stability studies

Accelerated stability studies of Pemetrexed disodium microspheres at temperature 25±2ºC/60±5% RH as per ICH guidelines were studied for 90 days. The assays and appearance of samples were determined as a function of the storage time. There was no change in the physical appearance, particle size was not change significantly and assay was found to be 82.4 % after 90 days. From the data, it is observed that there was negligible change in the drug content indicating chemical stability. The results of stability data has shown in below table 6.

Table 6: Accelerated stability (25±2°C / 60±5% RH) data of Pemetrexed disodium microspheres.

Test	0 days	15 days	30 days	45 days	60 days	75 days	90 days
Description	**White to almost white**	**White to almost white**	**White to almost white**	**White to almost white**	**White to almost white**	**White to almost white**	**White to almost white**
Assay of F7 formulation	82.6%	81.7%	80.4%	79.5%	77.8%	76.3%	75.4%
Assay of F9 formulation	83.9%	83.4%	82.4%	81.7%	80.3%	79.6%	78.2%
Assay of F10 formulation	86.5%	86.3%	85.5%	84.4%	83.6%	83.3%	82.4%

CONCLUSION

In the present study, attempts were made to prepare pemetrexed disodium microspheres for controlled release by double emulsion solvent evaporation technique using PLGA 50:50 polymer. The selection of organic solvent, concentration of polymer, speed of primary and secondary homogenization were found to have played a predominant role in the preparation. The formed microspheres were found to be uniform and spherical in shape. The optimized formulations exhibited 95.6% *in vitro* controlled release for one month. From the experimental results it is evident that the controlled release microspheres of pemetrexed disodium can be successfully formulated for subcutaneous administration in the treatment of patients with pleural mesothelioma and non-small cell lung cancer.

REFERENCES

1. Targeted and controlled drug delivery by S.P Vyas, R. K. Khar, 425-441.
2. Encyclopedia of pharmaceutical technology, 1998; 10: 1-30.
3. Navin Dixit, Sheo Dutt Maurya, Bhanu P.S.Sagar. *Sustained Release Drug Delivery System*. Indian Journal of Research in Pharmacy and Biotechnology, May-June 2013; 1(3): 305-310.
4. Debjit Bhowmik, Harish Gopinath, B. Pragati Kumar, S. Duraivel, K. P. Sampath Kumar. *Controlled Release Drug Delivery Systems*. The Pharma Innovation, 2012; 1(10): 24-32
5. Alagusundaram. M, Madhu Sudana Chetty. C, Umashankari.K, Attuluri Venkata Badarinath, Lavanya. C and Ramkanth. S. *Microspheres as a Novel Drug Delivery System - A Review*. International Journal of ChemTech Research, July-Sept 2009; 1(3): 526-534.

6. Prasanth V.V, Akash Chakraborthy Moy, Sam T Mathew, Rinku Mathapan. *Microspheres - An Overview*. International Journal of Research in Pharmaceutical and Biomedical Sciences, Apr – Jun 2011; 2(2): 332-338.
7. Kataria Sahil, Middha Akanksha, Sandhu Premjeet, Ajay Bilandi and Bhawana Kapoor. *Microsphere: A Review*. International Journal of Research in Pharmacy and Chemistry, IJRPC, 2011; 1(4): 1184-1198.
8. Ketie Saralidze, Leo H. Koole and Menno L.W. Knetsch. *Polymeric Microspheres for Medical Applications*. Materials, 2010; 3: 3537-3564.
9. Ramteke K.H, Jadhav V.B, Dhole S.N. *Microspheres: as carrieres used for novel drug delivery system*. IOSR Journal of Pharmacy (IOSRPHR), July 2012; 2(4): PP 44-48.
10. Freiberg S, Zhu XX. *Polymer microspheres for controlled drug release*. National Center for Biotechnology Information, Int J Pharm, 2004 Sep 10; 282(1-2): 1-18.
11. Wu XS, Wang N. *Synthesis, characterization, biodegradation, and drug delivery application of biodegradable lactic/glycolic acid polymers*. National Center for Biotechnology Information, J Biomater Sci Polym Ed, 2001; 12(1): 21-34.
12. S. S. Bansode, S. K. Banarjee, D. D. Gaikwad, S. L. Jadhav, R. M. Thorat. *Microencapsulation : A Review*. International Journal of Pharmaceutical Sciences Review and Research, March – April 2010; 1(2): 008, 38-43.
13. Yoon Yeo and Kinam Park. *Control of Encapsulation Efficiency and Initial Burst in Polymeric Microparticle Systems.* Archibes of Pharmacal Research, Arch Pharm Res, 2004; 27(1): 1-12.
14. Ram Chand Dhakar, Sheo Datta Maurya, Bhanu PS Sagar, Sonia Bhagat, Sunil Kumar Prajapati, Chand Prakash Jain. *Variables Influencing the Drug Entrapment Efficiency of Microspheres: A Pharmaceutical Review*. Scholars Research Library, Der Pharmacia Lettre, 2010; 2(5): 102-116.
15. F. O. M. S. Abreu, M. M. C. Forte, T. B. L. Kist, L. P. Honaiser. *Effect of the preparation method on the drug loading of alginate-chitosan microspheres*. eXPRESS Polymer Letters, 2010; 4(8): 456–464.
16. Navin Dixit, Sheo Dutt Maurya, Bhanu P.S.Sagar. *Sustained Release Drug Delivery System*. Indian Journal of Research in Pharmacy and Biotechnology, May-June 2013; 1(3): 305-310.
17. Bhargava Ankit, Rathore R.P.S, Tanwar Y.S., Gupta S, Bhaduka G. *Oral Sustained Release Dosage Form: An Opportunity to Prolong the Release of Drug*. International

Journal of Advanced Research in Pharmaceutical and Bio Sciences, IJARPB, 2013; 3(1): 7-14.

18. L. Mary Sony Philo, Sumit Shah, A.V Badarinath, C.Gopinath. *Formulation and Evaluation of Octreotide Acetate Loaded PLGA Microspheres*. International Journal of Pharmacy and Pharmaceutical Sciences, Int J Pharm Pharm Sci, 5(3): 615-621.
19. Sigimol Joseph , B. Arya, Dinu Chacko, Eldho Jacob, Mahitha Mathew, Sharon Anna Babu, SS. Anju. *Formulation and Evaluation of Diclofenac Sodium Loaded Albumin Microspheres*. International Journal of Research in Pharmaceutical and Nano Sciences, 2014; 3(1): 6-11.
20. Santosh Voruganti, J. Surya Chandra Padman. Formulation and Evaluation of BSA Loaded PLGA Microparticles. International Journal of Pharmaceutical Sciences And Research, IJPSR, 2013; 4(3): 1013-1021.
21. Patel Samir kumar, Tara Chand and Talsania Maulik. *Formulation Development and Evaluation of Microspheres Containing Duloxetine Hydrochloride*. International Journal of Research in Pharmaceutical and Biomedical Sciences, Apr– Jun 2013; 4(2): 568-572.
22. Hiren kumar K. Makadia and Steven J. Siegel. *Poly Lactic-co-Glycolic Acid (PLGA) as Biodegradable Controlled Drug Delivery Carrier*. Polymers, 2011; 3: 1377-1397.
23. Nastaran Nafissi Varcheh, Vera Luginbuehl, Reza Aboofazeli and Hans Peter Merkle. *Preparing Poly (Lactic-co-Glycolic Acid) (PLGA) Microspheres Containing Lysozyme-Zinc Precipitate Using a Modified Double Emulsion Method*. Iranian Journal of Pharmaceutical Research, Iran J Pharm Res, 2011 Spring; 10(2): 203–209.
24. Alireza Mahboubian, Seyyed Kazem Hashemein, Shadi Moghadam, Fatemeh Atyabi, and Rassoul Dinarvand. *Preparation and In vitro Evaluation of Controlled Release PLGA Microparticles Containing Triptoreline*. Iranian Journal of Pharmaceutical Research, Iran J Pharm Res, 2010 Autumn; 9(4): 369–378.
25. Srujan Peddi, K. Narendra Kumar Reddy, Sumit Shah, K. Sravani Shilpa and K. Madhuri. *Preparation and Characterization of Sustained Release PLGA- Loaded Microparticles Containing Leuprolide Acetate*. International Journal of Pharmaceutical, Chemical and Biological Sciences, IJPCBS, 2013; 3(4): 1200-1207.
26. Ankit D. Patel, Shalin K. Parikh, Dr. D. J. Sen and Dr. C. N. Patel. *Development and Validation of High Performance Liquid Chromatographic and UV Spectrophotometric Method for Estimation of Pemetrexed Disodium in Bulk Drug and Pharmaceutical Formulation*. International Journal of Drug Development & Research, Int. J. Drug Dev. & Res, April-June 2011; 3(2): 301-307.

27. Shilpa Sappidi, Kiran Thadkala, Jagannath Kota and Jithan Aukunuru. *Preparation and characterization of ethyl cellulose microspheres encapsulating Metformin hydrochloride and Glipizide*. Scholars Research Library, 2014; 6(4): 213-226.
28. Sandeep kumar, Namrata Gautam, Dr. Md. Akram Minhaj, Sana, Sapna Kapoor. *Development and Optimization of Lyophilization Cycle.* World Journal of Pharmaceutical Research, 4(2): 1053-1062.
29. Pavani Sriram, Deepak Kamlekar, Sujitha Hazari. *Preparation and In Vitro Evaluation of Chitosan Microspheres of Eplerenone*. International Journal of Pharmacy and Pharmaceutical Sciences, Int J Pharm Pharm Sci, 5(3): 226-229.
30. Gautam Singhvi, Mahaveer Singh. *Review: In-Vitro Drug Release Characterization Models.* International Journal of Pharmaceutical Studies and Research, IJPSR, January-March, 2011; II(I): 77-84.