IJPSR (2015), Vol. 6, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 17 September, 2014; received in revised form, 05 November, 2014; accepted, 11 January, 2015; published 01 May, 2015

FORMULATION AND CHARACTERIZATION OF PARENTERAL IN SITU IMPLANTS OF TARIQUIDAR BIMESYLATE

R. Bindu [1], Prathima Srinivas *[1], D S Ravindrababu [2]

Sri Venkateshwara College of Pharmacy [1], Madhapur, Telengana, India
Celon Laboratories Limited [2], Hyderabad, Telengana, India.

Keywords:

In situ implants,
Tariquidar bimesylate, PLGA,
Sustained release, parenteral depot

Correspondence to Author:
Dr. Prathima Srinivas
(M. Pharm Ph. D)
Principal, Sri Venkateshwara College of Pharmacy, Hitech City Road
Madhapur, Hyderabad- 500081
Telengana, India.

E-mail:drpssvcp@gmail.com

ABSTRACT: The objective of present study was to formulate and evaluate Tariquidar bimesylate subcutaneous in situ implants in order to minimize the frequency of doses and toxicity and to improve the therapeutic efficacy. The anthranilic acid derivative, tariquidar (XR9576), is a potent and selective P-gp inhibitor being developed clinically for the treatment of multidrug resistant tumors. Tariquidar bimesylate in situ implants were prepared by polymer precipitation method using two different grades of polymer poly (Lactide -co-glycolide) (PLGA) with three different concentrations. The preparation involves dissolving the biodegradable polymer poly (Lactide -co-glycolide) PLGA 85:15 and PLGA 75:25 in N-methyl-2- pyrrolidone. To this solution drug was added and the polymer drug solution was injected in to the aqueous mediato forma solid implant. Based on initial burst release, optimized formulations were selected and evaluated for surface morphology, *in vitro* drug release and accelerated stability studies. It was observed that the formulations with more polymer concentration and in combination with polyethylene glycol(PEG) and Benzy l benzoate (BB) showed increased entrapment efficiency and exhibited moderate burst release and sustained release for 156 hrs. Release kinetics was calculated for optimized formulations and the formulation F10, F11, F12, F13 followed Higuchi kinetics with nonfickian diffusion. Optimum use of various polymers along with benzyl benzoate and polyethylene glycol can result in better sustained release for Tariquidar bimesylate and can be explored for therapeutic benefit in Multidrug resistant tumors.

INTRODUCTION: Drug resistance is a major impediment to chemotherapy in many human cancers. P-glycoprotein (P-gp), encoded by the *MDR-1* gene, is an energy-dependent efflux pump that lowers the intracellular concentrations of a variety of chemotherapeutic agents [1, 2]. Expression of the *MDR-1* gene at levels found in many clinical tumor samples can confer multidrug resistance *in vitro*, suggesting *MDR-1*/Pgp–mediated drug resistance is clinically relevant [3].

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.6(5).2028-34

Article can be accessed online on:
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.6(5).2028-34

The anthranilic acid derivative, tariquidar (XR9576), is a potent and selective third generation P-gp inhibitor being developed clinically for the treatment of multidrug resistant tumors [4, 5]. At low concentrations tariquidar restores the sensitivity of many multidrug-resistant human tumor cell lines in vitro by inhibiting Pgp-mediated drug efflux Polymeric drug delivery systems are attractive alternatives to control the release of drug substances to obtain defined blood levels over a specified time [6, 7, 8].

The patients suffer from some disease conditions such as heart disorders, osteoporosis, tumors, and often benefit from such long term drug delivery systems due to improved patient compliance.In-situ

implants are advantageous over the microspheres and implants. Microspheres manufacturing process is often complex and difficult to control [9]. There are also questions with regard to costs and batch-to-batch product uniformity. In solid implants, they require surgical implantation or the use of large troches to administer the product. Hence the patient compliance is an issue. In situ implants have more patient and physician acceptance related to ease of administration, reliable kinetic profiles and product costs. Injectable in situ forming implants are classified in to five categories, according to their mechanism of depot formation: (1) thermoplastic pastes, (2) in situ cross linked systems, (3) insitu polymer precipitation, (4) thermally induced gelling systems and (5) insitu solidifying organ gels. Of these, in situ polymer precipitation systems have become commercially available so far.

The Solvents commonly used in this approach include N-methyl-2-pyrrolidone, Polyethylene Glycol (PEG-4000), Dimethyl sulfoxide (DMSO) and Benzylbenzoate. The insituforming implant systems have several advantages compared to traditional pre formed implants systems. Due to their injectable nature, implant placement is less invasive and painful for the patient thereby improving compliance.

Currently, only two FDA approved products are in the market utilizing this type of system, Eligard® poly (Lactide-co-glycolide) and Atridox®. Eligard®, using the atrigel delivery system and marketed by Sanofi-aventis in the US (Medigene in Europe),is a subcutaneously injected implant that releases leupraloid acetate over a period of 3 months to suppress testosterone levels for prostate cancer treatment. Atridox® is another ISFI system that also uses the Atrigel delivery systems to deliver the antibiotic agent, Doxycyclin to sub-gingival space to treat periodontal disease. Some disadvantages of in situ implants are high burst release, potential solvent toxicity and high viscosity of the polymeric solution.

MATERIALS AND METHODS:

Materials: Tariquidar bimesylate was obtained from Anthem Biosciences pvt limited, Ltd., poly (Lactide-co-glycolide) PLGA-75:25 (RG755S), poly (Lactide-co-glycolide), PLGA-85:15 (RG855S) and PLGA 50:50 (RG504) was obtained from Evonikroehmgmbh, Germany. All solvents were HPLC grade and were obtained from Merck chemicals, Mumbai

PREFORMULATION STUDY:

Solubility studies of Tariquidar bimesylate:

The solubility of Tariquidar bimesylate was determined in the solvents Dimethyl sulphoxide, N-Methyl pyrrolidine, Ethanol, Methanol, Water for injection, Dichloromethane, polyethylene glycol and Benzylbenzoate. This was accomplished by adding excess drug Tariquidar bimesylate to each solvent of interest and allowing the mixtures to shake for 24 hours. All the mixtures were then centrifuged, and a known volume of supernatant was removed from each mixture. The amount was determined by using UV-Visible spectrophotometer (PerkinElmer's) at 240 nm

TABLE 1: SOLUBILITY OF TARIQUIDAR BIMESYLATE IN DIFFERENT SOLVENTS

Solvent	Quantity (mg/ml)
DMSO	200
NMP	200
N,N Dimethyl Acetamide	200
Methanol	100
Benzyl benzoate	5
Polyethylene glycol	5
Water for injection	2.5
Ethanol	2
Dichloromethane	0.833

DMSO – Dimethyl sulfoxide; NMP -N-Methyl pyrrolidine

Solubility studies of Polymer:

The solubility of PLGA 50:50, PLGA75:15, PLGA85:15 and PLA was determined from in different solvents such as Dimethyl sulphoxide, N-Methyl 2-pyrrolidone as shown in **Table 2.**

TABLE 2: SOLUBILITY OF POLYMERS IN DIFFERENT SOLVENTS

Polymer	Solvent	Solubility (mg/ml)
PLGA 50:50	NMP	500
	DMSO	66.66
PLGA 75:25	NMP	>300
	DMSO	50
PLGA 85:15	NMP	200
PLA	NMP	Not soluble

mg/mL – milligram/milliliter

Determination of λ max of Tariquidar bimesylate: The UV absorption spectrum of Tariquidar bimesylate in water for injection shown

in **Fig.1.** A solution of Tariquidar bimesylate containing concentration 5µg/ml was prepared in Water and UV spectrum was taken using a PerkinElmer's double beam spectrophotometer and scanned between 200 to 400 nm. The maxima obtained in the graph were considered as λmax for the drug Tariquidar bimesylate. The compound exhibited maximum at 240nm in water.

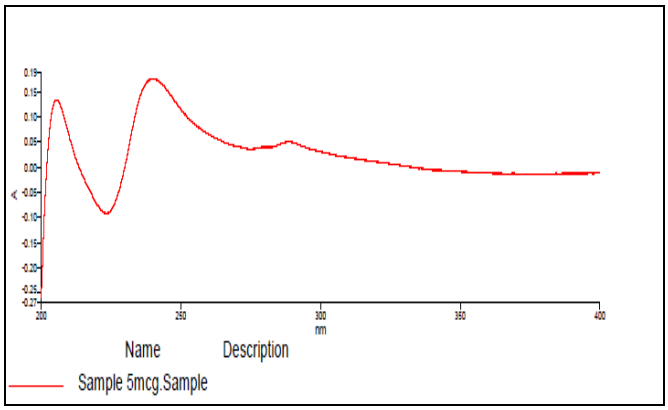


FIG 1: TARIQUIDAR BIMESYLATE λ MAX IN WFI

Method of preparation of implants:

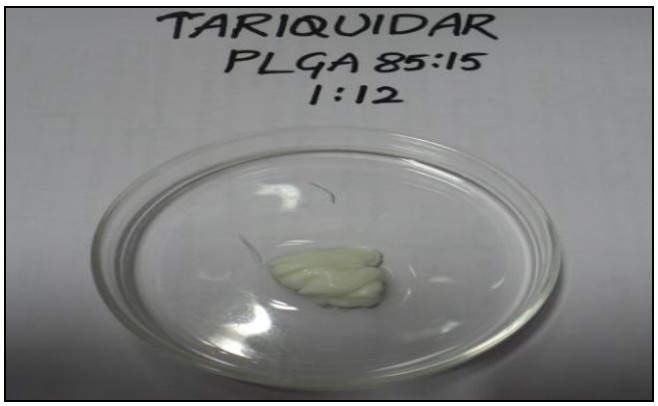
In situ implants of Tariquidar bimesylate were prepared by polymer precipitation method developed by Atrixl® laboratories and is designated as Atrigele technology [7]. Different formulations were prepared using 27-33% of polymer PLGA which was dissolved in organic phase containing 1.5 ml N-methyl-2-pyrrolidone in glass vials until a clear solution was formed. To this 25 mg of Tariquidar bimesylate was added. The polymer-drug solution was stirred vigorously (for 2 hrs) until clear solution was formed [8].

This solution was filled in syringes, and gradually injected in to Water for Injection at 37^0 C with 18 gauge needle for the formation of implants. Within 5-10 minutes solid round implants formed and were evaluated. Various formulations were prepared using 3 different grades of polymers as shown in **Table 3**. In situ implants formed after injecting into water as shown in **Fig 2**.

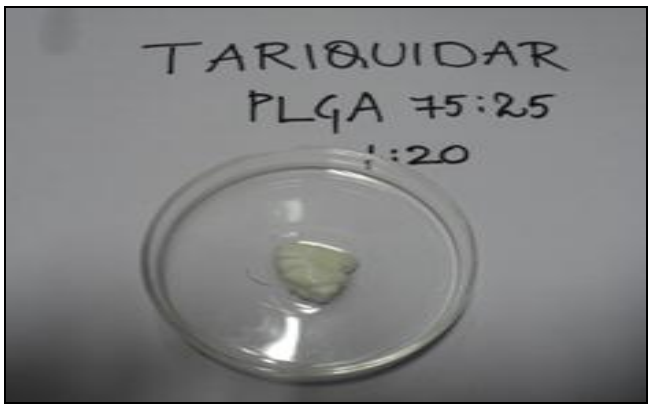
TABLE 3: FORMULATION OF TARIQUIDAR BIMESYLATE IN SITU IMPLANTS BY USING THREE DIFFERENT GRADES OF POLYMER

Batch code	Qty. API (mg)	PLGA 85:15 (mg)	PLGA 75:25 (mg)	PLGA 50:50 (mg)	Benzyl Benzoate (ml)	Poly ethylene Glycol (ml)	NMP (mg)	Drug/ polymer ratio	Injection volume (ml)
F1	25	300	-	-	-	-	800	1:12	1.2
F2	25	400	-	-	-	-	800	1:16	1.5
F3	25	500	-	-	-	-	800	1:20	1.5
F4	25	-	300	-	-	-	800	1:12	1.5
F5	25	-	400	-	-	-	800	1:16	1.2
F6	25	-	500	-	-	-	800	1:20	1.5
F7	25	-	-	300	-	-	800	1:12	1.0
F8	25	-	-	400	-	-	800	1:16	1.2
F9	25	-	-	500	-	-	800	1:20	1.5
F10	25	300	-	-	0.25	-	800	1:12	1.2
F11	25	300	-	-	-	0.25	800	1:16	1.2
F12	25	-	500	-	0.25	-	800	1:20	1.5
F13	25	-	500	-	-	0.25	800	1:20	1.5

mg – milligram;mL - milliliter



A. TARIQUIDAR BIMESYLATE INSITU IMPLANT WITH PLGA 85:15



B. TARIQUIDAR BIMESYLATE INSITU IMPLANT WITH PLGA 75:25

FIG 2: INSITU IMPLANTS FORMED AFTER INJECTION

Characterization of Implants:

Optical microscopy:

Shape and surface morphology of insitu implants was studied using Optical microscopy. Optical microscopic studies were carried out by placing dry implants on optical microscope brass stub. These studies were carried out on initial day, 10^{th} day and 30^{th} day so as to observe the degradation of polymer

FTIR studies:

FTIR spectra were recorded for Tariquidar bimesylate and Formulation. The characteristic peaks of Tariquidar bimesylate were compared with the peaks obtained for formulation.

Determination of Free drug content/Initial burst release of drug:

After injecting the drug-polymer solution into water for injection, the container was kept aside until a spherical shaped implant was obtained. Then the solution was replaced with fresh medium. The resultant solution was filtered and analyzed for the free drug content and initial burst release at 240nm by using UV-Visible spectrophotometer.

***In vitro* drug release:**

In vitro drug release studies were performed using dialysis membrane with WFI. In situ implants were placed in conical vials open on one side and closed with dialysis membrane on other side. The formulations were placed in a 50 ml Water for injection at 37^0C. At different time intervals, 5 ml samples were withdrawn and replaced with fresh medium and the withdrawn samples analyzed for drug content by UV-visible spectrophotometer at 240nm. After every one week the complete medium was withdrawn and replaced by fresh medium to avoid saturation of the medium. The obtained data was fitted in to mathematical equation (zero order, first order, higuchi model) in order to describe the kinetics and mechanism of drug release from the implant formulations.

***In vitro* release kinetics:**

The plots of cumulative percentage drug release v/s. time, cumulative percent drug retained v/s. root time, and log cumulative percent drug retained v/s. time and log cumulative percent drug release v/s. log time were plotted. The slopes and the regression co-efficient (r^2) were calculated

Accelerated Stability studies:

To assess the physical and chemical stability of the Tariquidarbimesylate formulations, accelerated stability studies were conducted for 3 months. The optimized formulations were placed in vials and stored at 40^0C/75%RH. After 90 days the formulations was checked for physical appearance and drug content using High Performance Liquid Chromatography (HPLC).

RESULTS AND DISCUSSION:

Solubility studies:

The solubility studies of Tariquidar bimesylate in different solvents revealed that it was freely soluble in DMSO and NMP. In ethanol it was slightly soluble

Formulation optimization:

Formulations were prepared using three different grades of polymer with (PLGA 85:15, PLGA 75:25 and PLGA 50:50) different concentrations of polymers with a solvent NMP. The compositions and ratios of in situ implants were listed in **Table 3**.

Formulations before injection in to buffer were found to be clear and transparent. Upon injection of polymer solutions in to the water for injection, the polymer solidified as the solvent dissipated in to aqueous medium and formed implants. The various formulations prepared using different polymer concentrations are shown in **Table 3**. Based on burst release, out of 13 formulations six formulations were selected as optimized for further evaluation as shown in **Table 4**.

TABLE 4: OPTIMIZED FORMULATIONS OF TARIQUIDAR BIMESYLATE

S.No	Batch code	Qty. API (mg)	PLGA 85:15(mg)	PLGA 75:25(mg)	Benzyl Benzoate(mL)	Poly ethylene Glycol (mL)	NMP (mg)	Drug/ polymer ratio	Injection volume (mL)
1	F1	25	300	-	-	-	800	1:12	1.2
2	F6	25		500	-	-	800	1:20	1.5
3	F10	25	300	-	0.25	-	800	1:12	1.5
4	F11	25	300	-	0.5	-	800	1:12	1.5
5	F12	25	-	500	-	0.25	800	1:20	1.2
6	F13	25	-	500	-	0.5	800	1:20	1.5

mg – milligram;mL - millilite

Optical microscopy:

The Morphology and surface appearance of in situ implants were examined by using Optical microscopy. The microscopic studies were carried out on various formulations on initial day, 10th day and after 30 days. The image of F10 formulation on initial day was shown in **Fig 3** indicates fewer pores on the surface. The **Fig 4** and **5** shown increase in pore size indicating the degradation of the polymer

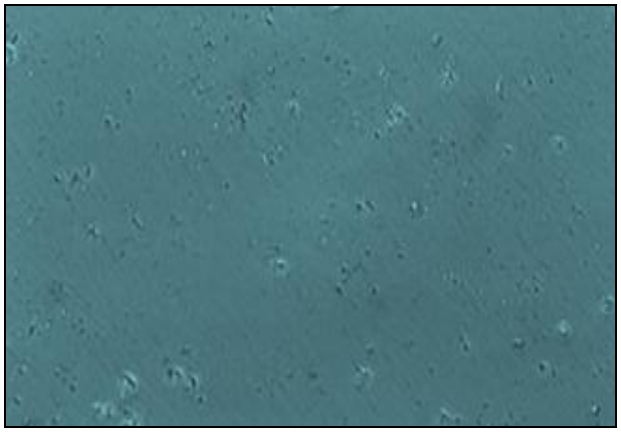


FIG 3. F10 MICROSCOPIC IMAGE ON INITIAL DAY

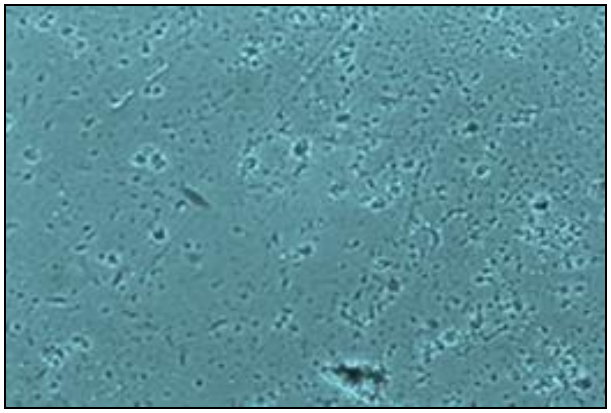


FIG 4. F10 MICROSCOPIC IMAGE ON 10TH DAY

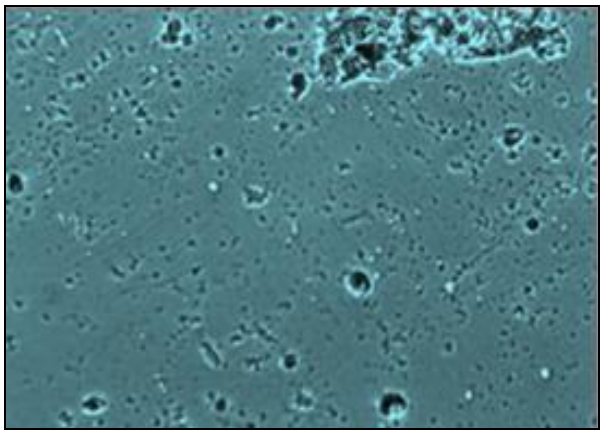


FIG 5. F10 MICROSCOPIC IMAGE AFTER 30 DAYS

FTIR Studies:

FTIR spectra obtained for Tariquidar bimesylate, physical mixture and formulation presented in the **Fig 6** and **7**. The characteristic peaks of Tariquidar bimesylate were compared with the peaks obtained for formulation. The FTIR spectra revealed that there were no interactions between polymer and Tariquidar bimesylate

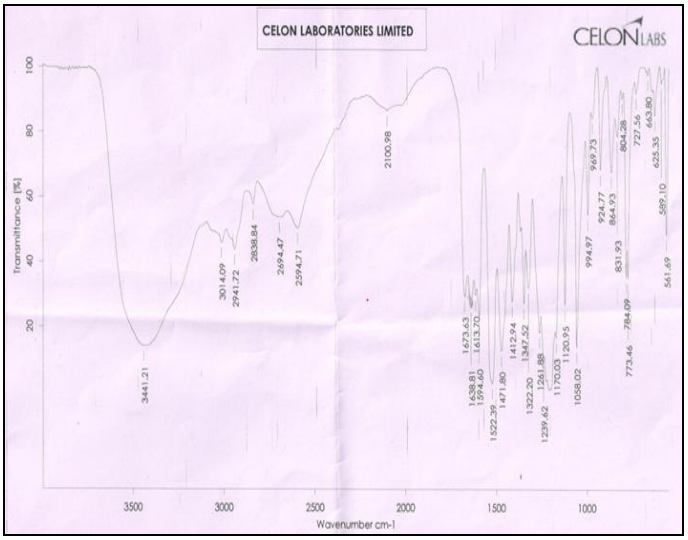


FIG. 6: FTIR OF PURE API AND PHYSICAL MIXTURE

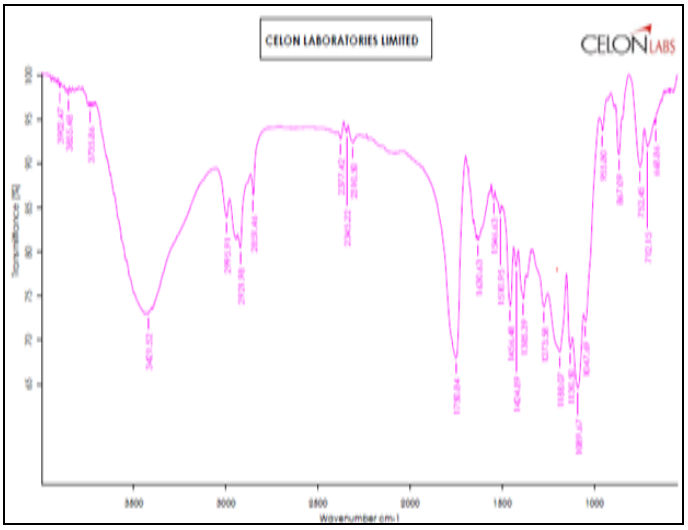


FIG. 7: FTIR OF OPTIMIZED FORMULATION F10

Free drug content/Initial burst release of drug:

Percentage encapsulation efficiency and free drug content of formulated Tariquidar bimesylate implants was shown in **Table 5** and **Fig 8**. F1, F6 formulation showed minimum encapsulation efficiency where as F10, F11, F12 and F13 formulation showed maximum entrapment efficiency**.**

TABLE 5: FREE DRUG CONTENT AND ENTRAPMENT EFFICIENCY OF DIFFERENT FORMULATIONS

Formulations	% Entrapment Efficiency	% Free Drug
F1	70	30
F6	61	39
F10	82	18
F11	85	15
F12	84	16
F13	83	17

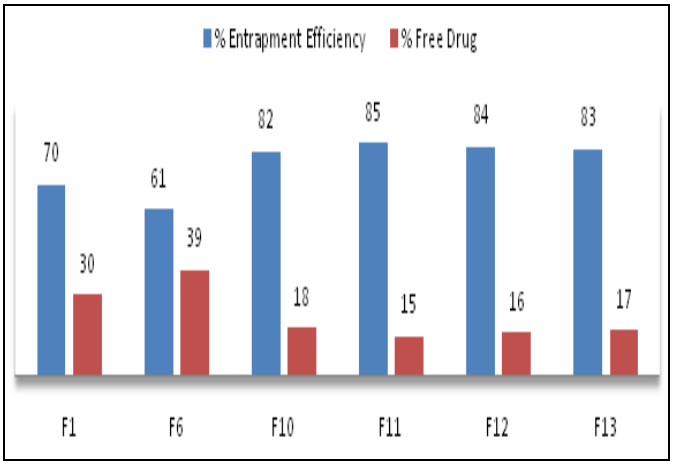


FIG 8: GRAPHICAL REPRESENTATION OF ENCAPSULATION EFFICIENCY AND FREE DRUG OF FORMULATED TARIQUIDAR BIMESYLATE IN SITU IMPLANTS

In vitro drug release:

In vitro drug release studies were performed using dialysis membrane with WFI. Comparison of *in vitro* release studies of various formulations are shown in **Fig 9, 10, 11, 12**. As the polymer concentration is decreased, more burst release was observed. More prominent burst release was observed in case of F1 and F6 formulations. The release was found to be almost similar in all optimized formulations. Formulations containing benzyl benzoate and PEG showed sustained release nearly up to 6 days (156 hours) when compared to conventional formulation. Hence benzyl benzoate and polyethylene glycol can be used in order to avoid initial burst drug release and to sustain the drug release from implants

In vitro release kinetics:

The slopes and the regression co-efficient (r2) were listed in **Table 6**. The co-efficient values indicated that formulations F1, F6 follows zero order release and formulations F10, F11, F12, F13 follows Higuchi kinetics. Higuchi equation explains the diffusion controlled release mechanism. The diffusion exponent 'n' values of korsemeyer-peppas model for formulation F1, F6 was found to be in the range of 0.8-1 indicating case II transportand formulations F11, F11, F12, F13 found to be in range of 0.45-0.8 indicating non fickian diffusion of Tariquidar bimesylate from in situ implants.

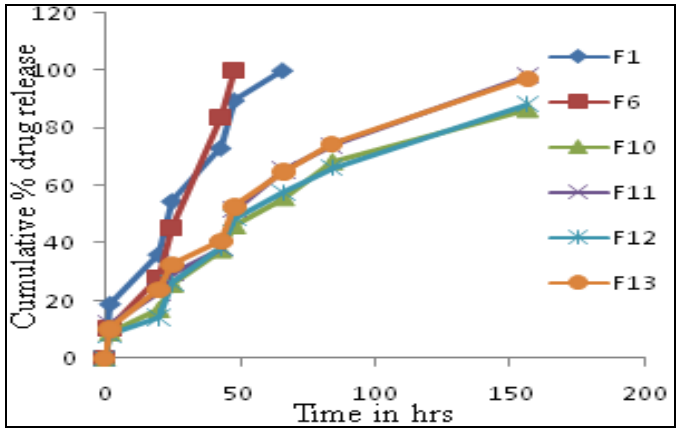


FIG. 9: ZERO ORDER RELEASE GRAPH

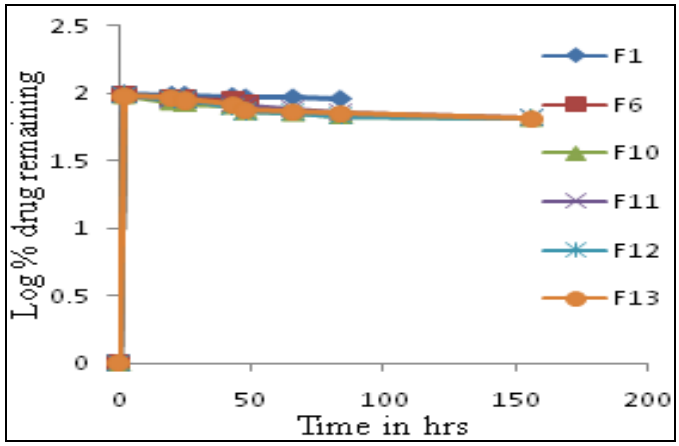


FIG. 10: FIRST ORDER RELEASE GRAPH

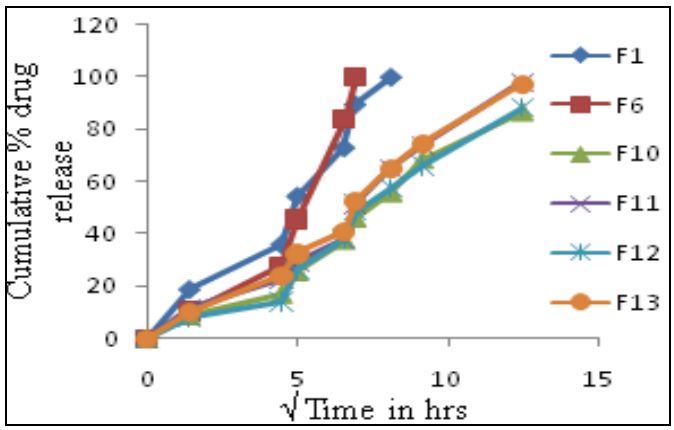


FIG. 11: HIGUCHI RELEASE GRAPH

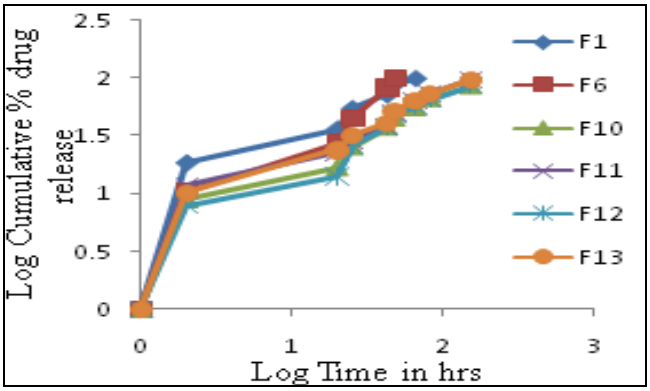


FIG. 12: KORSEMEYER PEPPAS RELEASE GRAPH

Accelerated Stability studies:

Accelerated stability studies of Tariquidar bimesylate formulation (F10) at temperature 40^0C/75%RH were studied for 90 days. The formulation was a clear yellow color solution. No color change indicates physical stability for 3 months. The drug content was analyzed and data is presented in **Table 7**. From the data, it was observed that there was negligible change in the drug content indicating chemical stability.

TABLE 6: REGRESSION COEFFICIENT AND DIFFUSION COEFFICIENT VALUES

Formulations	Zero order		First order		Higuchi kinetics		Korsemeyer-peppas	
	K_0	R^2	K_1	R^2	K_H	R^2	n	R^2
F1	1.480	0.959	0.011	0.222	12.14	0.954	0.886	0.823
F6	1.972	0.972	0.021	0.293	13.37	0.869	0.981	0.894
F10	0.573	0.914	0.004	0.095	7.291	0.961	0.772	0.903
F11	0.622	0.924	0.004	0.093	8.052	0.961	0.766	0.874
F12	0.573	0.914	0.004	0.094	7.436	0.956	0.787	0.911
F13	0.616	0.915	0.003	0.092	8.066	0.973	0.783	0.895

TABLE 7: RESULTS OF STABILITY TESTING OF TARIQUIDAR BIMESYLATE FORMULATION

	F10			
Test	Initial	1 month	2 months	3 months
Description	Yellow colored solution	Yellow colored solution	Yellow Colored solution	Yellow colored solution
pH	Between 7.00 and 9.50	7.86	7.83	7.80
Assay	81.9%	81.45%	80.97%	80.97%

CONCLUSION: Tariquidar bimesylate Insitu-implants for controlled release by polymer precipitation method were prepared using PLGA 85:15 and PLGA 75:25 polymers can be successfully formulated. Optimum use of various polymers along with benzyl benzoate and polyethylene glycol can result in better sustained release and can be explored for therapeutic benefit in Multidrug resistant tumors.

ACKNOWLEDGEMENT: The authors acknowledge Celon Laboratories, Hyderabad, India for gift sample of PLGA.

REFERENCES:

1. Tiwari AK, Sodani K, Dai CL, Ashby CR Jr, Chen ZS Revisiting the ABCs of multidrug resistance in cancer chemotherapy. Curr Pharm Biotechnol 2011; 12: 570–594. doi: 10.2174/138920111795164048
2. Hopper-Borge EA, Churchill T, Paulose C, Nicolas E, Jacobs JD, et al. Contribution of Abcc10 (Mrp7) to in vivo paclitaxel resistance as assessed in Abcc10 mice. Cancer Res2011; 71: 3649–3657. doi: 10.1158/0008-5472.can-10-3623
3. Borel F, Han R, Visser A, Petry H, van Deventer SJ, et al. Adenosine triphosphate-binding cassette transporter genes up-regulation in untreated hepatocellular carcinoma is mediated by cellular microRNAs. Hepatology2012; 55: 821–832. doi: 10.1002/hep.24682
4. Kannan P, Shukla S, Ambudkar SV, Pike VW, Halldin C, et al. The "specific" P-glycoprotein inhibitor tariquidar is also a substrate and an inhibitor for breast cancer resistance protein (BCRP/ABCG2). ACS Chem Neurosci 2011; 2: 82–89. doi: 10.1021/cn100078a
5. Sun Y-L, Chen J-J, Kumar P, Chen K, Sodani K, et al. Reversal of MRP7 (ABCC10)-Mediated Multidrug Resistance by Tariquidar. PLoS ONE 2013; 8(2): e55576. doi:10.1371/ journal. pone. 005557
6. Y.S.P.C.W.D.P.P.M.H.M. Rhee. Sustained-Release Injectable Drug Delivery Systems. 2012.
7. S.S. Lee, P. Hughes, A.D. Ross, and M.R. Robinson Biodegradable implants for sustained drug release in the eye, Pharm. Res., 2010; 27 2043-2053.
8. M.K. Joo, M.H. Park, B.G. Choi, and B. Jeong Reverse thermogelling biodegradable polymer aqueous solutions, Journal of Materials Chemistry, 2009; 19, 5891-5905.
9. C. Wischke and S.P. Schwendeman Principles of encapsulating hydrophobic drugs in PLA/PLGA microparticles, Int. J. Pharm., 2008; 364, 298.

How to cite this article:
Bindu R, Srinivas P and Ravindrababu DS: Formulation and Charecterization of Parenteral In Situ Implants of Tariquidar Bimesylate. Int J Pharm Sci Res 2015; 6(5): 2028-34.doi: 10.13040/IJPSR.0975-8232.6(5).2028-34.