IJPSR (2017), Volume 8, Issue 9 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 03 February, 2017; received in revised form, 24 April, 2017; accepted, 27 May, 2017; published 01 September, 2017

FORMULATION AND *IN VITRO* CHARACTERIZATION OF ANTICANCER DRUG LOADED SOLID LIPID NANOPARTICLES

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Keywords:

Temozolomide,
Solid lipid nanoparticles,
In vitro drug release kinetics

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ABSTRACT: The aim of the study was to prepare Temozolomide loaded solid lipid nanoparticles and evaluating loaded solid lipid nanoparticles. Temozolomide loaded solid lipid nanoparticles were prepared by micro emulsification method using different lipid (stearic acid) concentration keeping drug concentration unchanged, poloxamer 188 and cetyl alcohol as surfactant and co-surfactant. The prepared SLN were characterized for surface morphology by SEM analysis, drug loading and % entrapment efficiency, FTIR, zeta potential and *in vitro* diffusion studies. The lowest and highest % Entrapment Efficiency was found to be 85.85% and 97.45% for F1 to F5 batches respectively. Release studies were done by using saline phosphate buffer pH 7.4 using dialysis bag diffusion method. Zeta potential was found to be - 20.3 mV and particle size was found to be 119.34. For 8 hrs *in vitro* drug release found to be 79.16%, 74.45%, 71.42%, 61.36%, and 55.13% respectively. The release data was analysed by different kinetic models and found that the formulations best fit model is peppas and the drug release mechanism was Fickian diffusion (n-value 0.3808).

INTRODUCTION: Cancer chemotherapy is now established value and highly specialized field and complete re-emission should be the goal of this therapy in which the drugs are often used in maximum tolerated doses. Intensive regimens used earlier yield better results. Cancers were treated with one drug at a time [1]. The main problem of cancer therapy is not the lack of efficient drugs, but that these drugs are very difficult to concentrate in the tumour tissue without leading to toxic effects on neighbouring organs or tissues [2]. So now days going for targeted drug delivery systems *i.e.* nanoparticles. The uncontrolled and often rapid proliferation of cells can lead to either a benign or malignant tumor [2, 3].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(9).3808-12

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

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (9).3808-12

Nanoparticles are solid colloidal particles ranging in size from 1 to 1000 nm and composed of macromolecular materials [4]. Solid lipid nanoparticles were defined as oil in water emulsion for parenteral nutrition, but the liquid lipid (oil) of the emulsion has been replaced by a solid lipid *i.e.* yielding solid lipid nanoparticles.

Solid Lipid Nanoparticles Provide the Following Advantages:

- Control and target drug release
- Improves the stability of pharmaceuticals
- High and enhanced drug content when compared to other carriers
- Feasibility of carrying both lipophilic and hydrophilic drugs
- Water based technology
- Easy to scale-up and sterilize
- Good biocompatibility
- Low toxicity
- SLNs particularly those in the range of 120-200 nm are not taken up readily by the cells of the

reticulo-endothelial system and thus bypass liver and spleen filtration [4, 5].

The main advantage of the study is to provide drug delivery by oral route to the targeted site. SLN are colloidal lipid systems, which have been proposed for several administration routes, such as parenteral, oral and topical. These solid lipid nanopaticles were prepared by different methods as following

- High shear homogenization
- Hot homogenization
- Cold homogenization
- Ultrasonication/high speed homogenization
- Solvent emulsification/evaporation
- Micro emulsion based SLNs preparations
- SLNs preparation by using supercritical fluid
- Spray drying method
- Double emulsion method
- Hot homogenization followed by ultrasonication [6-8].

Temozolomide: It is an oral alkylating agent which can be used for the treatment of Grade IV astrocytoma an aggressive brain tumor, also known as glioblastoma multiforme as well as Melanoma, a form of skin cancer. It is having protein binding about 15% and having 100% bioavailability, metabolism is spontaneously hydrolyzed at physiologic pH to the active species, 3-methyl -(triazen-1-yl) imidazole-4-carboxomide (MTIC) and to Temozolomide acid metabolite and half life is 1.8 hours [9, 10]. The objective of the present research was to study the preparation of loaded solid lipid nanoparticles of Temozolomide by micro-emulsification technique and to study the effects of composition of lipid materials and surfactant mixture on the particle size, zeta potential, drug entrapment efficiency, FTIR, SEM and *in vitro* drug release.

MATERIALS AND METHODS:

Materials: Temozolomide, stearic acid from SD fine chem. Limited, Mumbai, poloxamer 188 as a git sample from MSN Laboratories, hyd and cetyl alcohol as gift sample from trident pharmaceuticals, Pondicherry and all other ingredients were analytical grade.

Methods:

Preformulation Study: The drug-excipient interaction studies were performed by suitably mixing the drug with the chosen additives and stored the mixture for 4 weeks. The drug excipient mixture was evaluated periodically for their physical properties like TLC, FTIR and physical observations and compared with the drug raw material observations. Calibration curve of the drug was estimated in saline phosphate buffer pH 7.4 and the suitably diluted solutions were analyzed by spectro photometrically using double beam UV spectro photometer at 266.8 nm. The estimation was performed in triplicate and the regression co-efficient was derived to ensure the linearity of the calibration curve.

Preparation of Solid Lipid Nanoparticles: SLN were prepared from a warm w/o/w micro emulsion containing Stearic acid as lipid, poloxamer 188 as surfactant, and cetyl alcohol as co-surfactant.

TABLE 1: COMPOSITION OF TMZ LOADED SOLID LIPID NANOPARTICLES

Formulation	Molar : ratio (Drug: Lipid)	Drug (mg)	Lipid (mg)	Surfactant (mg)	Co-surfactant (mg)
F-1	1:0.	500	200	150	500
F-2	1:1.2	500	400	200	500
F-3	1:1.7	500	600	250	500
F-4	1:2.2	500	800	300	500
F-5	1:2.7	500	1000	350	500

Temozolomide SLN was prepared by micro emulsification technique. Stearic acid was first heated to 75 °C to ensure total melting and then Temozolomide was dispersed in it. Double-distilled water was heated separately (at least 5 °C above the melting point of the lipid). Poloxamer 188 and cetyl alcohol were added to the hot water under constant stirring and allowed to equilibrate. Next, the aqueous surfactant solution was added to the lipid phase while stirring in magnetic stirrer and homogenised at rpm of about 1000 for 2 hrs. The homogenised product represents oil in water emulsion, which on filtering and drying it forms solid lipid nanoparticles.

Characterization of Solid Lipid Nanoparticles:

Fourier Transform Infra-Red Spectroscopy (FT-IR) Analysis: Drug and lipid compatibility was studied by infrared spectroscopy using Avatar Thermo Nicolet FT-IR spectrophotometer. The spectra was recorded in the wavelength region of 400-4000 cm^{-1} for drug, lipid and Temozolomide, after mixing respective samples with dried KBr powder and compressing to a disc by a hydraulic press at 5 t compression.

Determination of Drug Loading and % Entrapment Efficiency: Equivalent weight of prepared nanoparticles was dissolved in a mixture of methanol and chloroform (5:5). Require dilutions were performed with the same solvent mixture and analyzed in a UV-Visible spectrophotometer at 266.8 nm against the blank.

$$\text{Drug loading} = \frac{\text{Amount of drug present in nanoparticles}}{\text{Total amount of lipid nanoparticles}} \times 100 \quad \text{-----(1)}$$

Amount of drug present in nanoparticles = concentration of dug × dilution factor

$$\% \text{ Entrapment efficiency} = \frac{\text{Practical drug loading}}{\text{Theoretical drug loading}} \times 100 \quad \text{----- (2)}$$

Particle Size, Surface Morphology and Zeta Potential: The surface morphology (roundness, smoothness, and formation of aggregates) and particle size were studied by scanning electron microscopy (SEM). Zeta potential of the best formulation was determined by zeta metre.

Evaluation of *In vitro* Drug Release: *In vitro* drug release was evaluated by using a dialysis bag diffusion technique. Equivalent weight of nanoparticles were packed in dialysis bag and placed in basket immersed in 900 ml of saline phosphate buffer pH 7.4 as dissolution medium at 37 °C paddle rotating at 75 rpm. An aliquote amount of 5 ml sample was drawn in a time interval of 30, 60, 90,120, 180, 240, 360 and 480 min and replaced with fresh dissolution medium. Dilutions to be done if necessary and absorbance to be recorded at 266.8 nm.

***In vitro* Drug Release Kinetics:** In order to investigate the mechanism of release, the release data were analysed with the following mathematical models: zero-order kinetic (Eq. 3), first-order kinetic (Eq. 4) and Higuchi kinetic (Eq. 5)

$$Q = K_0 t \quad \text{----- (3)}$$

$$\text{Log } Q_t = \text{Log } Q_0 + \frac{K1t}{2.303\ t} \quad \text{------(4)}$$

$$Q = K_H t^{1/2} \quad \text{----- (5)}$$

The following plots were made: Qt versus t (zero-order kinetic model), log (Q_0–Q_t) versus t (first-order kinetic model) and Q_t versus $t^{1/2}$ (Higuchi model), where Q_t is the percent of drug released at time t, Q_0 is the initial amount of drug present in the lipid nanoparticles and K_0, K_1 and K_H are the rate constants of the equations 3, 4 and 5, respectively.

korsmeyer-peppas model and Hixson crowell model were applied to interpret the drug release kinetics from the formulations. Based on the highest regression values (r^2) for correlation coefficients for formulations, the best fit model was decided.

RESULTS AND DISCUSSION:

Drug Polymer Interaction by FT - IR Analysis: Identification of Temozolomide was done by FTIR. Drug excipient interaction studies showed that there is no any physicochemical interaction between the drug and the selected additives of formulations. The results were shown in **Table 2**.

TABLE 2: INTERPRETATION AND COMPARISON OF FTIR PEAKS

Functional groups	Stretching / Deformation	Drug (cm^{-1})	Drug + Stearic acid (cm^{-1})	Drug + polaxomer188 (cm^{-1})
C=O	Stretching	1729.59	1727.28	1734.68
C – N	Stretching	852.56	853.20	844.68
C=N	Stretching	1597.35	1597.95	1598.06
C=C	Stretching	1675.23	1675.82	1678.08
CH_3 – N	Stretching	2874.95	2852.98	2877.11
N – H	Stretching	3384.11	3385.06	3386.19
CH_3	Stretching	2971.49	2924.96	2952.24

Drug Loading and % Entrapment Efficiency: Many different drugs had been incorporated in SLNs. The prerequisite to obtain a sufficient loading capacity was a sufficiently high solubility of the drug in the lipid melt. Relative higher drug EE % was one of the major advantages of SLNs. The drug content in five batches of Temozolomide solid lipid nanoparticles was studied. The amount of drug present in TMZ solid lipid nanoparticles was determined in each batch. **Table 3** shows the results of the drug entrapment efficiency in each of these formulations. It was observed that the entrapment efficiency increased with the increase in concentration of lipid in the formulations. The maximum entrapment was found in F-5 of 97.45% and lowest entrapment in F-1 of 85.85%.

TABLE 3: DRUG LOADING AND % ENTRAPMENT EFFICIENCY OF TMZ LOADED SLN

Formulation	Drug Loadind	% Entrapment Efficiency
F-1	50.5%	85.85%
F-2	40.90%	90%
F-3	33.33%	91.05%
F-4	29.62%	94.8%
F-5	26.33%	97.45%

Particle Size, Surface Morphology and Zeta Potential: Average particle size of TMZ loaded solid lipid nanoparticles was found to be 119.34 nm.

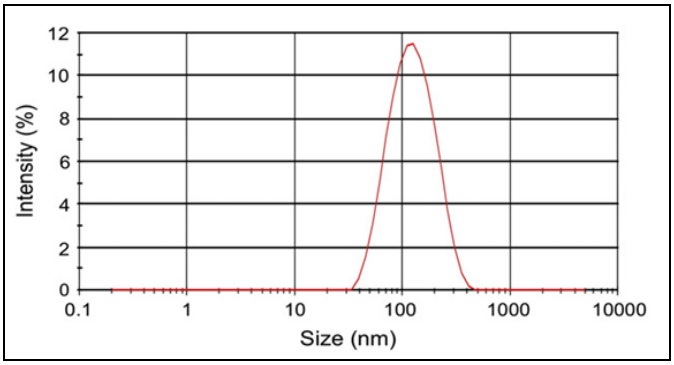

FIG. 1: PARTICLE SIZE DISTRIBUTION OF TMZ LOADED SLN OF F-2

Surface morphology was done by using instrument JEOL-JSM-T330A. Magnification was done at 7500- 20000X for taking photographs. Shown in **Fig. 2**.

From surface morphology studies it was revealed that the nanoparticles were spherical in shape. Surface charge analysis of the TMZ loaded solid lipid nanoparticles was done by Malvern Zetasizer and zeta potential was found to be -20.3mV which indicates they are slightly stable. The **Fig. 3** was shown below.

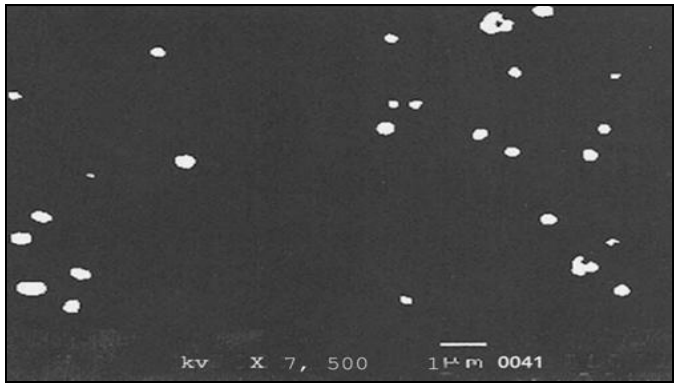

FIG. 2: SCANNING ELECTRON MICROSCOPY OF F-2

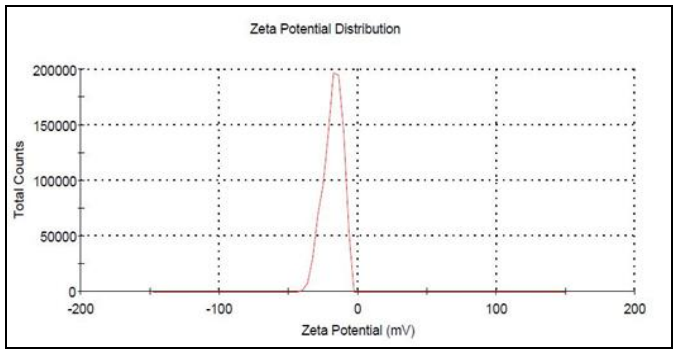

FIG. 3: ZETA POTENTIAL OF TMZ LOADED SLN OF F-2

***In vitro* Drug Release Studies:** *In vitro* release of all batches of Temozolomide loaded SLN (F1-F5) were carried out in saline phosphate buffer pH 7.4 and the formulation showed the release 55.13%, 61.36%, 71.42%, 74.45% and 79.16% respectively at the end of 8 hrs. Among all the formulations F2 showed good controlled release and able to release entire amount in 12 hrs. Therefore it may be considered as the optimized formulation. The dissolution profiles of all the formulations were shown in **Fig. 4.**

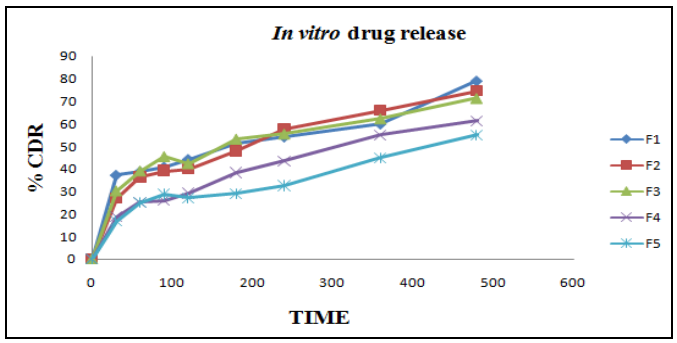

FIG. 4: *IN VITRO* RELEASE PROFILE OF TMZ LOADED SOLID LIPID NANOPARTICLES OF F1 - F5

***In vitro* Drug Release Kinetic Studies:** Data obtained from *in vitro* release studies of prepared TMZ loaded solid lipid nanoparticles were fitted to various kinetic models such as zero order, first order, Higuchi model, Hixson Crowell cube root and korsmeyer-Peppas model. The formulation F2 showed best fit in korsmeyer-Peppas model release and with the r^2 value 0.9891 and n = 0.3808 which obeys Fickian diffusion. The consolidated dissolution data analysis of all the formulations is shown in **Table 4** and drug release model of TMZ - SLN F2 is shown in **Fig. 5.**

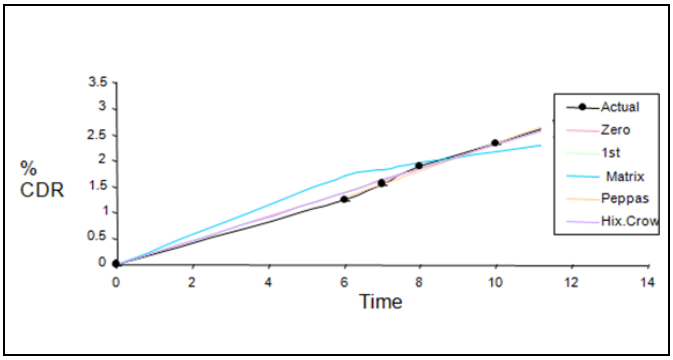

FIG. 5: CUMULATIVE PERCENTAGE RELEASE PROFILE WITH MODEL FITTING OF FORMULATION F-2

TABLE 4: KINETIC VALUES OBTAINED FROM *IN VITRO* RELEASE DATA OF DIFFERENT TEMOZOLOMIDE SOLID LIPID NANOPARTICLES

S. No	Best fit model	Zero order r^2	1st order r^2	Matrix r^2	Korsmeyer-Peppas r^2	Hix.crow. r^2	Korsmeyer-Peppas n
F-1	Peppas	0.4034	0.8598	0.9311	0.9416	0.7737	0.2701
F-2	Peppas	0.6652	0.9200	0.9836	0.9891	0.8616	0.3808
F-3	Peppas	0.2647	0.7830	0.9372	0.9844	0.6746	0.3049
F-4	Matrix	0.8357	0.9501	0.9962	0.9888	0.9215	0.4684
F-5	matrix	0.7608	0.8939	0.9720	0.9545	0.8594	0.3963

ACKNOWLEDGEMENT: Nil.

CONFLICT OF INTEREST: There is no conflict of interest.

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How to cite this article:
Bandi UM, Philip K, Reddy DB, Swaroopa A, Prabakaran L and Parthasarathy G: Formulation and *in vitro* characterization of anticancer drug loaded solid lipid nanoparticles. Int J Pharm Sci Res 2017; 8(9): 3808-12.doi: 10.13040/IJPSR.0975-8232.8(9).3808-12.

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