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FORMULATION AND EVALUATION OF ORAL TIO_2 NANOCARRIERS OF IMATINIB MESYLATE

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ABSTRACT

In the present research work an attempt has been made for the formulation of oral nanocarriers for Imatinib mesylate. Imatinib is proved to be highly efficient in the treatment of chronic myeloid leukaemia and gastrointestinal stroma tumor. Imatinib loaded Titanium dioxide nanocarriers were prepared by solvent emulsification and evaporation method. The prepared formulation was evaluated for drug–carrier compatibility, drug entrapment studies and *In vitro* drug release studies. FTIR studies have shown no interaction between the drug and the $nTiO_2$ of various proportions (1:1, 1:2, 2:1, and 3:4). The encapsulation efficiency was noted as 60.02%, 85.46%, 79.19% and 89.92% for ratios 1:1, 1:2, 2:1 and 3:4. The drug release rates were 7.24%, 25.88%, 17.16%, 98.13%.The best formulation was 3:4 ratio and drug release was 98.14% at 24 h. The release of drug from nanocarriers was found to follow the Higuchi diffusion mechanism. The drug diffuses from solid nanocarriers at a comparatively slower rate as the distance for diffusion increases.

INTRODUCTION:

Nanomedicine, the application of nanotechnology in medicine, aims to overcome problems related to diseases at the nano scale where most of the biological molecules exist and operate. It is an emerging field with wide range of applications from diagnosis to therapy, which includes targeted delivery and regenerative medicine. The role of nanotechnology in cancer is quite significant, enhancing the earlier crude procedures with modern diagnosis and therapeutic strategies. Cancer nanotechnology is an interdisciplinary field of research that is based on biology, chemistry, engineering and medicine and is aiming at a giant leap in cancer diagnosis and treatment (Wang et al., 2010).

Targeted drug delivery seeks to concentrate the medication in the tissues of interest while reducing the relative concentration of the medication in the surrounding tissues. This improves the efficacy of the drug while reducing side effects. Drug targeting delivers drugs exclusively to receptors, organs, or any other specific part of the body to which one wishes to deliver them. Multi functionalized single walled carbon nanotubes were used for targeting transporters (Yang et al., 2008). Inorganic nanoparticles are relatively stable over broad ranges of temperature and pH, yet their lack of biodegradation and slow dissolution raises safety questions, especially for long term administration. Imatinib is a cancer medication prescribed to treat leukemia and gastrointestinal

tumors. It operates by inhibiting proteins associated with cancer cell growth in order to relieve symptoms, prevent the spread of cancer cells, and aid other treatments. Imatinib is one of the newest anticancer drugs in the market and was one of the first drugs to be pushed through Food and Drug Administration's (FDA) fast track designation for approval. The drug is designed to inhibit tyrosine kinases such as Bcr-Abl and is used in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stroma tumor. The Chemical name of Imatinib Mesylate is 4-4[(4-methyl-1- piperazinyl) methyl]-N-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl] amino] phenyl] – benzamide mono methane sulfonate. It has a molecular formula of $C_{29}H_{31}N_7O.CH_4O_3S$ and a molecular weight of 589.71. It has the structural formula (Fig.1). Imatinib Mesylate is a white crystalline powder which is freely soluble in distilled water, 0.1 N HCl, methanol and sparingly soluble in dimethyl ether (Martindale et al., 2009).

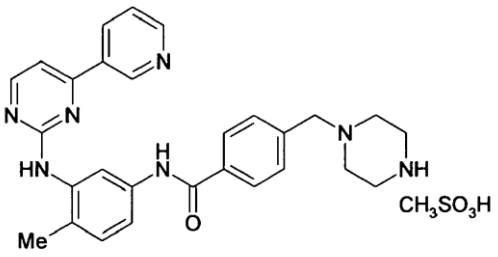

Fig. 1. Structure of Imatinib mesylate

MATERIALS AND METHODS:

Imatinib mesylate was received as gift sample form MSN Laboratories, Hyderabad, TiO2 nanopowder was purchased from SRL, India and all other chemicals used were of analytical grade.

Standard graph of imatinib mesylate:

100mg of Imatinib mesylate was accurately weighed and transferred to 100ml volumetric flasks it was dissolved in few ml of methanol (5ml) and diluted to volume with 0.1N HCL to give a stock solution 1000µg/ml. From stock solution 10ml was withdrawn and volume was made up to 100ml to give 100µg/ml (Priya et al., 2013). From this solution dilutions were done (20, 40, 60, 80, 100 µg/ml) and volume was made up to 10ml by using 0.1N HCL. The absorbance of solutions was measured against 0.1N HCL as blank at 281nm using UV- spectrophotometer.

Compatibility studies:

FT-IR spectra of freeze-dried nanoparticles were obtained with Bruker Vertex-70 spectrophotometer using potassium bromide (KBr) disk technique. NPs size distribution and zeta potential were determined using photon correlation spectroscopy with a Zetasizer 3000 (DelsaNano S, USA). The size distribution analysis was performed at a scattering angle of 90° at a temperature of 25°C (Hamid et al., 2006) and the zeta potential (Feng et al., 2004) was measured by electrophoretic light scattering using a disposable zeta cuvette. All measurements were performed in triplicate and the results were presented as mean ± SD. The morphological examination of nanoparticles was performed on scanning electron microscopy (SEM) (JEM-2010HR, Japan). The nanoparticles were applied to a carbon-coated 300 copper grid and then were stained with 2% phosphotungstic acid for viewing by SEM.

Entrapment efficiency and drug loading Capacity:

NPs were dissolved in HPLC grade methanol by stirring for 4h, and the solution was ultrasonicated for 5 minutes (Anil et al., 2002). This colloidal dispersion was filtered by 0.22µm membrane filter. The concentration of free drug in the solution was determined by UV-VIS Spectrophotometry (LAB INDIA) using a wavelength of 281 nm. The amount of drug in nanoparticles was calculated as the difference between the total amount drug linked with nanoparticles and the amount present in the supernatant. Freshly prepared drug loaded nanoparticles were used for determining the drug-loading capacity. The Entrapment efficiency and Loading capacity of nanoparticles were determined in triplicate and calculated as follows:

$$EE\ (\%) = \frac{(\text{Total amount of drug} - \text{Free drug})}{\text{Total amount of drug}} \times 100 \quad \textbf{(1)}$$

$$DLC\ (\%) = \frac{(\text{Total amount of drug} - \text{Free drug})}{\text{Total amount of nanopartic les}} \times 100 \quad \textbf{(2)}$$

Where EE= Encapsulation Efficiency,

DLC= Drug Loading Capacity

DOSAGE FORM DESIGN:

Various amounts of drug and carriers are considered for formulation of drug loaded TiO_2 nanoparticles (Beija et al., 2012). In the dosage form designing various effects like amount of drug loading, effect of TiO_2 nanoparticles quantity, effect of solvent system ratio were studied (Table 1).

***In- vitro* drug release studies:**

The release of drug from nanoparticles was studied by dialysis method in 0.1N HCL buffer. 2ml samples were instilled in the dialysis bag which was screwed with two clamps at each end. The dialysis bag was dipped into the receptor compartment containing 35 ml of dissolution medium and stirred continuously at 100 rpm and maintained at 34°C. The receptor compartment was closed to prevent evaporation of the dissolution medium. Samples were withdrawn at regular time intervals, and the same volume was replaced with fresh dissolution medium. The samples were measured spectrophotometrically at 281nm.

Evaluation of drug kinetics:

Various models were tested for kinetics of drug release. To analyze the mechanism of the drug release rate kinetics of the dosage form, the obtained data were fitted into Zero order, First order, Higuchi and Korsmeyer-Peppas models (Peltier et al., 2006).The various release kinetic equations in which the experimental data can be fitted and drug release rate can be predicated as a function of some variable are mentioned below. The suitability of equation is judged on basis of best fit to the equation using statically indicators like R^2 value. To compare dissolution profile between two drug products model dependent statistic and model independent method can be used (Surakarta et al., 2010).

RESULTS AND DISCUSSION:

Calibration curve:

The maximum absorption of Imatinib mesylate in 0.1N HCL was scanned and found to have the maximum absorbance at 281nm. Standard graph of Imatinib mesylate in 0.1N HCL was plotted by taking concentration ranging from 2 to 10µg/ml and a good correlation was obtained with R^2 value of 0.998.

Compatibility studies:

The pure drug was analyzed using FTIR, the peaks were observed at 3345.32, 2094.81, 1636.01, 1456.89, 1181.13 cm^{-1} frequencies which indicated the presence of NH2, C≡C, C=C, C-C respectively and revealed the actual pure drug of Imatinib mesylate. After loading of the drug into nanoparticles the peaks of NH2, C≡C, C=C, C-C just shifted to the 3342.35, 2089.81, 1634.18 and 1179.01 cm-1 which are not shown considerable changes in the functional group shifting. The formulation has shown no incompatibility with TiO_2 particles (Fig. 3).

SEM analysis:

SEM analysis revealed that the morphology changes upon the linking of drug with TiO2 nanoparticles. The TiO2 nanoparticle (Fig. 4a) spherical structures are predominantly changed to network like structures on the particles upon the linking of the imatinib mesylate.

Drug release kinetics:

It was found that the *in-vitro* drug release from the best formulation (F1) of Imatinib loaded Titanium dioxide nanocarriers was best explained by zero order equation as the plot showed highest linearity (r = 0.987) followed by first order (r =0.934). So the release rate constant is concentration dependent. The release data was fitted into Higuchi's model (r= 0.974). So the drug diffuses at a comparatively slower rate as the distance for diffusion increases, which is referred to as square root kinetics (Higuchi's kinetics). Mechanism of drug release is explained by Korsmeyer-Peppas equation indicating a good linearity (r= 0.9762).

The release exponent 'n' was 0.5, which appears to indicate non-Fickian diffusion and may indicate that the drug release is controlled by more than one process, diffusion followed by erosion. The stability studies were performed at refrigerated temperature (2-8°C) and indicated no significant increase in particle size and zeta potential after one month of storage.

Table 1. Dosage form designing

Imatinib, (mg)	$nTiO_2$ (mg)	Ratio	Solvent system (methanol: water)
25	50	1:2	1:1
50	50	1:1	1:1
100	50	2:1	1:1
75	100	3:4	1:1
50	20	2:1	1:1
50	50	1:1	1:1
50	75	1:1.5	1:1
50	100	1:2	1:1
50	50	1:1	1:3
50	50	1:1	3:1
50	50	1:1	10ml water
50	50	1:1	10ml methanol

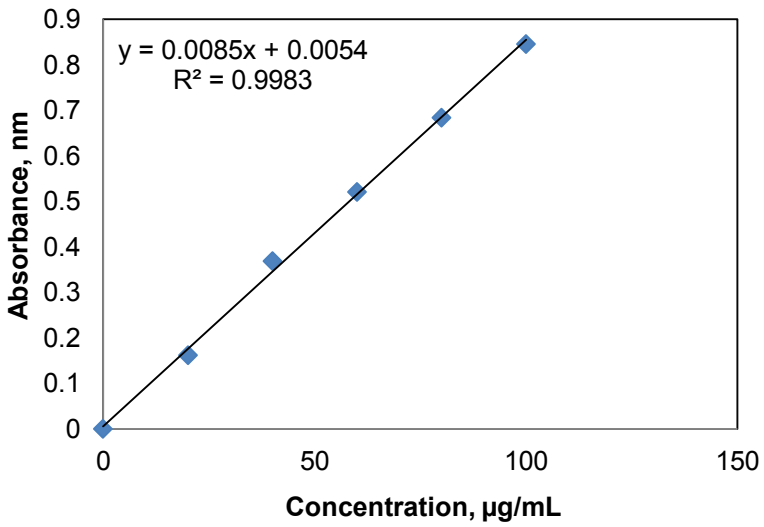

Fig 2. Standard Curve of Imatinib mesylate

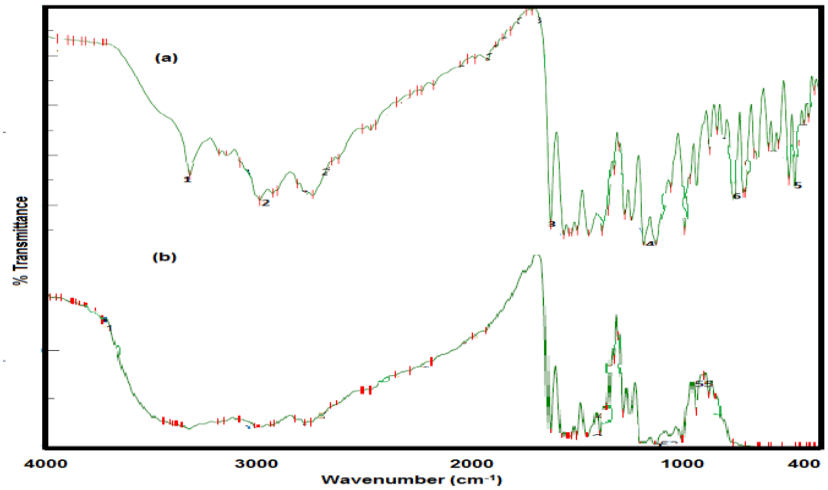

Fig 3. FTIR of pure drug Imatinib mesylate and formulated in TiO_2 nanoparticles

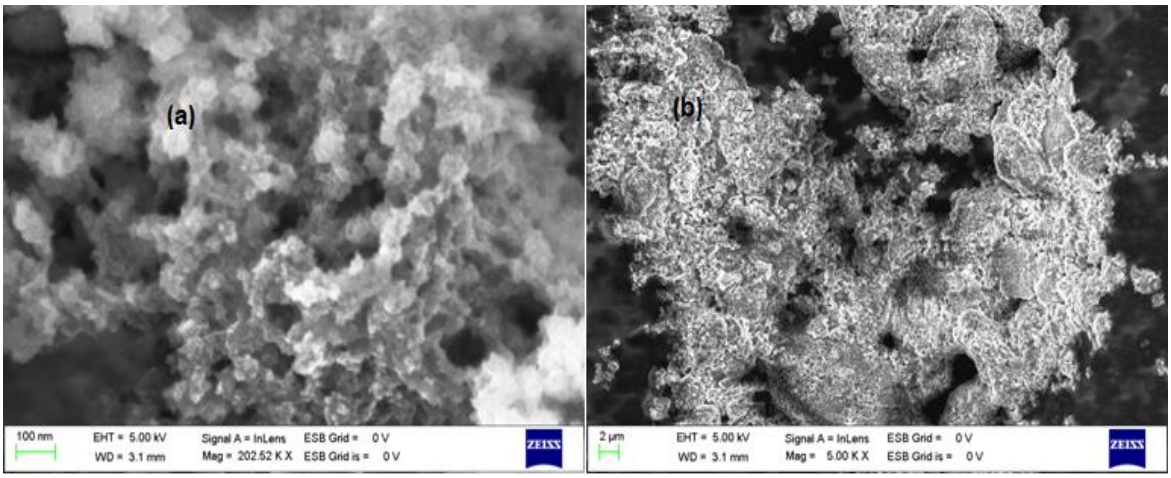

Fig. 4. SEM image of pure $nTiO_2$ and drug loaded $nTiO_2$

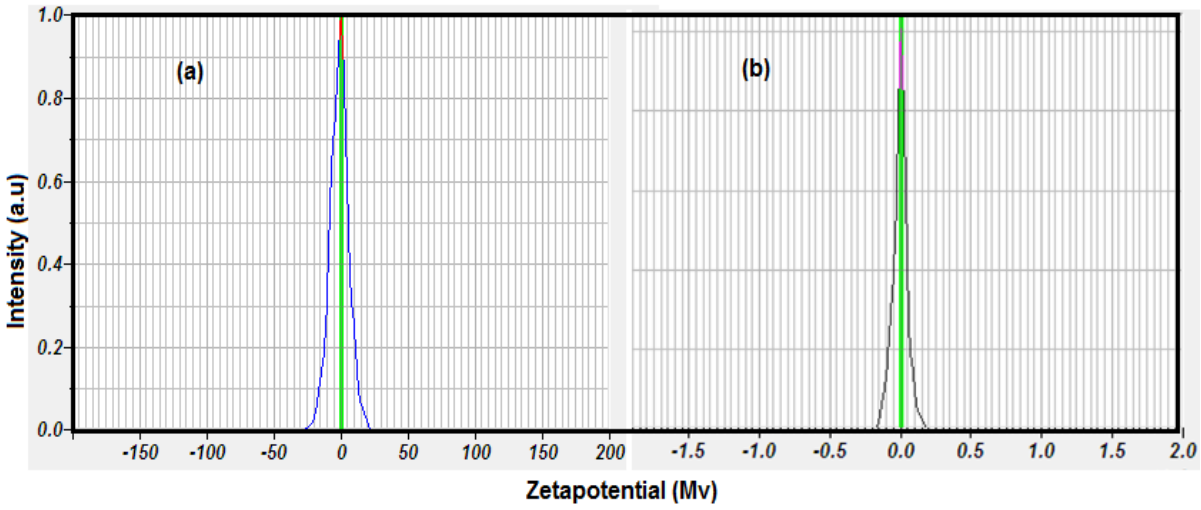

Fig. 5. Zeta potentials of pure $nTiO_2$ and drug loaded $nTiO_2$

Table. 2. Entrapment efficiencies of the various ratios of dosage forms

S.No.	Imatinib (mg)	$nTiO_2$ (mg)	Solvent sys-tem	EE (%)
1	25	50	1:1	78.46
2	50	50	1:1	60.20
3	100	50	1:1	85.19
4	75	100	1:1	89.92
5	50	25	1:1	58.5
6	50	75	1:1	59.5
7	50	50	1:1	52
8	50	50	1:3	54
9	50	50	3:1	62
10	50	50	10 mL water	50

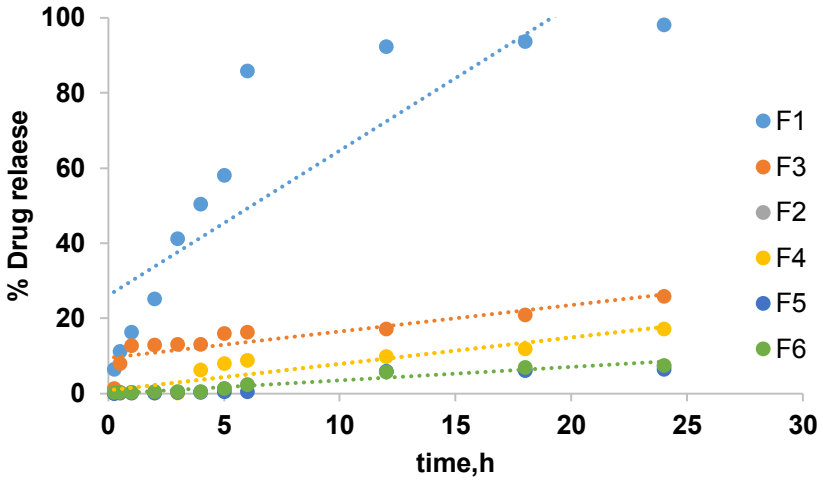

Fig.6. Drug release profiles of formulations of nanoparticles from F1-F6.

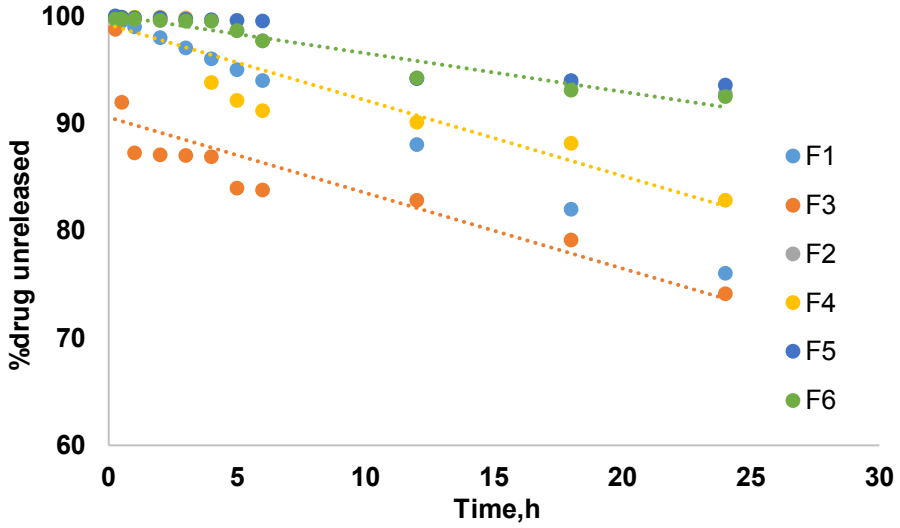

Fig. 7. First order release profiles of formulations of nanoparticles from F1-F6.

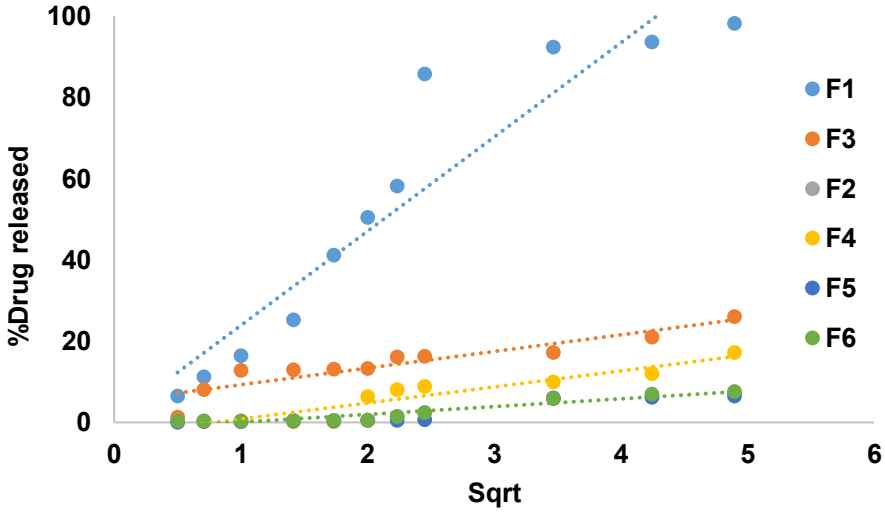

Fig. 8. Higuchi diffusion plot of nanoparticles of formulations F1-F6.

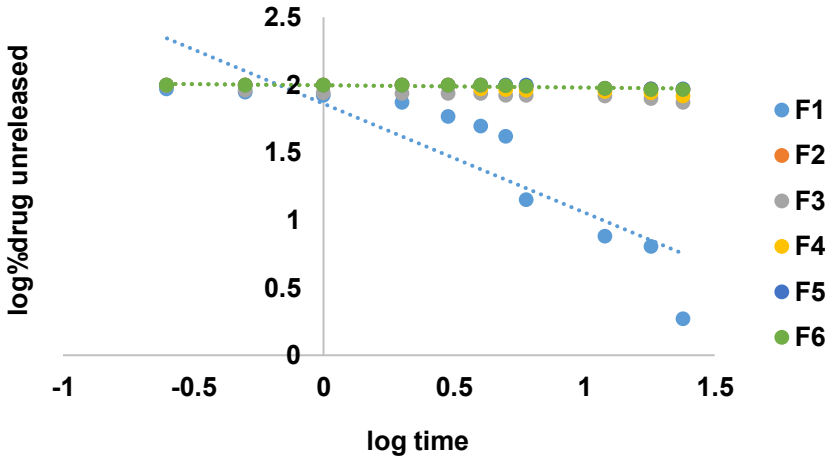

Fig. 9. Erosion plot of nanoparticles of formulations F1-F6

CONCLUSION

In conclusion,Imatinib loaded Titanium dioxide nano carriers were prepared by solvent emulsification and evaporation method. In-vitro characterization was carried out to evaluate the stability and release characteristics.From the above studies it was observed that formulation F1 has shown higher entrapment efficiency 89.92% which resulted in greater drug release of 98.13%. The equal concentration of nanoparticle and drug may be considered as optimum formulation. Nanoparticles were found to exhibit sufficient stability as evidenced by their zeta potential values and the release of drug trom the optimized formulation followed zero order.The solubility was increased by loading in TiO_2 nanoparticles and is intended to formulate capsule dosage forms. Further in-vivo studies are to be planned for observation of drug loaded TiO_2 nanoparticles behavior in biological systems.

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