Original Research Article

Design and evaluation of sorafenib tosylate nanoparticles including assessment of IC50 values using PC cell lines

ABSTRACT:

Introduction:

Sorafenib tosylate is an anticancer drug used for treatment of pancreatic cancer. In the present research work, Sorafenib tosylate is converted to nanoparticles with an aim to assess its anticancer activity with reduced concentration expecting less side effects of the parent drug.

Objective:

The aim of the present research work is preparation of nanoparticles of Sorafenib tosylate, evaluation at in-vitro level and to carry out promising nanoparticles for anti cancer activity for treatment of pancreatic cancer by MTT Assay method using PC cell lines including comparison of IC50 values of sorafenib tosylste nanoparticles and pure drug

Methodology:

The nanoparticles of sorafenib were prepared by salting out method using Eudragit S-100, sodium CMC and Zinc sulphate. Eight formulations were tried using varied drug to polymer ratios.

Results:

The promising formulation produced with drug to polymer ratio of 1:2 has particle size of 231.6nm and highest dissolution rate 76.2± 0.35% in 60 min and 82.5% in 90 min. These nanoparticles assessed by MTT assay method revealed reasonably reduced IC50 value of 0.848±0.217 compared to 1.92±0.14 in case of pure sorafenib tosylate.

Conclusion:

Sorafenib tosylate nanoparticles cabn be produced successfully by salting out method using drug to polymer (Sorafenib tosylate: Eudragit L-100) ratio of 1:3 by salting out method to possess ideal drug release characteristics. IC50 values of nanoparticles of sorafenib tosylate are reasonably reduced compared to pure drugs indicating very chances of reduced side effects with nanoparticles to treat pancreatic cancer effectively with reduced side effects.

KEY WORDS:

Sorafenib tosylate, nanoparticles, Salting out technique, anticancer activity, MTT assay technique, IC50.

1.INTRODUCTION:

Anti-cancer drugs prepared in the form of nanoparticles possess many therapeutic advantages including greater site-specific effect, high efficacy with less dose, less side effects to treat tumour cells [1,2]

Sorafenib tosylate (SRB) was approved by USFDA in December 2005, and received European commission marketing authorization in July 2006 for the use in the treatment of hepatocellular carcinoma that cannot be removed by surgery, renal cell carcinoma. It is one of the most preferred kinase inhibitor drug that formerly approved for therapy for primary kidney cancer (advanced renal cell carcinoma)[3]

It is a poorly water-soluble drug and commercially available as film coated tablets. The conversion to nanoparticles can be a promising approach to develop formulation suitable for oral or IV formulation due to expected rapid solubility, dissolution and bioavailability to treat pancreatic cancer[4] .Especially with the conversion of sorafenib tosylste to nanoparticles is expected to exhibit cytotoxic effect with much reduced concentration of active drug hence cause less side effects that are commonly possessed by chemotherapeutics.

Hence the main aim of the present research work is development and evaluation of nanoparticles of Sorafenib tosylate and to assess their activity for treatment of pancreatic cancer at ***in- vitro*** level by MTT Assay method using PC cell lines and to compare IC50 values of sorafenib tosylste nanoparticles and pure drug[5,6].

2.MATERIALS AND METHODS:

2.1.MATERIALS:

Sorafenib tosylate was obtained as gift sample from Aurobindo Pharmaceuticals, Hyderabad, Eudragit S-100 and sodium CMC were purchased from Merck Chemical Company (Mumbai, India), zinc sulphate and ethanol were obtained from Sigma-Aldrich (Mumbai, India). DMEM (Dulbecco's modified Eagles medium), MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide], trypsin, EDTA Phosphate Buffered Saline (PBS) and were purchased from Sigma Chemicals Co. (St. Louis, MO) and Fetal Bovine Serum (FBS) were purchased from Gibco. 25 cm^2 and 75 cm^2 flask and 96 well plated purchased from Eppendroff India.All other chemicals used in the study are of analytical grade.

2.2.Methods:

2.2.1.Preparation of nanoparticles of Sorafenib tosylate by salting out method:

Nanoparticles of sorafenib tosylate containing 200 mg of drug were prepared by salting out method. Various formulations were tried by changing composition and 8 no. of formulations (F1 to F8) produced with clear yield are presented in **Table 1.** During the preparation, Sorafenib tosylate and eudragit L-100 were dissolved in ethanol (organic phase). In another beaker, 2 gm of sodium CMC and 4gm of zinc sulphate were taken in 20 ml of distilled water and mixed well to dissolve completely (aqueous phase). Organic phase is suddenly poured in to aqueous part under stirring. Stirring is continued for about 3h under mechanical stirring for about 1500 rpm. After stirring a small quantity of water was added to the dispersion, mixed well and subjected for vacuum filtration. The filtrate was dried in Lyophillizer (Lyodel, JAPAN) for 24 hrs and the product was subjected to ***in-vitro*** evaluation.

Table 1: Composition of Sorafenib tosylate nanoparticles

Formulation	Drug (mg)	Eudragit S-100 (mg)	Sodium CMC (gm)	Zinc sulphate (gm)
F1	200	200	2.0	4.0
F2	200	300	2.0	4.0
F3	200	400	2.0	4.0
F4	200	500	2.0	4.0
F5	200	600	2.0	4.0
F6	200	700	2.0	4.0
F7	200	800	2.0	4.0
F8	200	250	2.0	4.0

2.2.2.In-vitro evaluation of nanoparticles of Sorafenib tosylate:

Prepared formulations, F1 to F8 are subjected to ***in-vitro*** evaluation by particle size determination, zeta potential measurement by using Zeta sizer, scanning electron microscopy, entrapment efficiency, ***in-vitro*** dissolution studies and drug-excipient interaction studies by FT-IR.

*2.2.2.1.*Drug content estimation:

Nanoparticles equivalent to contain 10 mg of sorafenib tosylate were weighed and taken in 100 ml beaker containing 50 ml of ethanol. The solution

was stirred at 1000rpm for 4 hrs in magnetic stirrer. The resultant solution was filtered and estimated for drug content using UV- visible spectrophotometer at λ_{max} at 224nm

2.2.2.2.In-vitro dissolution studies:

The drug release studies were carried out using USP XX1 dissolution testing type -II apparatus (Electrolab, INDIA) for prepared formulations F4 to F8 and for pure drug. The dissolution medium was pH 6.8 phosphate buffer maintained at temperature of 37±1º C with rotating speed of 100 rpm. At predetermined time intervals aliquot samples were withdrawn and diluted wherever necessary and analysed for drug content by UV spectrophotometer (Systronics, INDIA) λ_{max} at 265 nm. The volume withdrawn was replaced with fresh dissolution medium maintained at same temperature.

*2.2.2.3.*Particle size determination and zeta potential measurement:

Particle size and zeta potential of formulations F6 and F8 with enhanced % drug release values among all prepared formulations was determined by using zeta sizer (Horriba).

*2.2.2.4.*FTIR analysis:

The IR spectra of the samples were recorded for nanoparticles of SRB, F6 and for pure Sorafenib tosylate using a Fourier transform infrared spectrometer (Bruker, JAPAN). A small quantity of nanoparticles was mixed with 200mg of KBR and compressed to form pellets. These pellets were scanned in transmission mode in the spectral region 4000-400 cm-1 using a resolution of 4cm-1 and 32-co-added scans.

2.3.Assessment of anticancer activity by MTT assay technique:

2.3.1.Protocol:

2.3.1.1.MTT Assay Principle:

MTT Assay is a colorimetric assay that measures the reduction of yellow 3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The assay depends both on the number of cells present and, on the assumption, that dead cells or their products do not reduce tetrazolium.

The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble, dark purple colored formazan crystals. The cells are

then solubilized with a DMSO and the released, solubilized formazan reagent is measured spectrophotometrically at 570 nm.

2.3.1.2.Cell Line and Maintenance:

The Cancer cell lines were purchased from **NCCS, Pune and** the cells were maintained in MEM supplemented with 10 % FBS and the antibiotics penicillin/streptomycin (0.5 mL-1), in atmosphere of 5% CO 2 /95% air at 37^0 C.

2.3.1.3.Preparation of Test Compound:

For MTT assay, each test compound was weighed separately and dissolved in DMSO. Final concentration was made with medium and the cells were treated with series of concentrations from 1 to 5 µg/ ml.

2.3.1.4.Procedure:

Cell viability was evaluated by the MTT Assay with three independent experiments with six concentrations of compounds in triplicates. Cells were trypsinized and performed trypan blue assay to know viable cells in cell suspension. Cells were counted by hemocytometer and seeded at density of 5.0 X 10 3 cells / well in 100 µl media in 96 well plate culture medium and incubated overnight at 37 0 C. After incubation, take off the old media and add fresh media 100 µl with different concentrations of test compound in represented wells in 96 plates.

After 48 hrs., Discarded the drug solution and added the fresh medium with MTT solution (0.5 mg / mL-1) was added to each well and plates were incubated at 37 0 C for 3 hrs. At the end of incubation time, precipitates are formed as a result of the reduction of the MTT salt to chromophoreformazan crystals by the cells with metabolically active mitochondria. The optical density of solubilized crystals in DMSO was measured at 570 nm on a microplate reader. The percentage growth inhibition was calculated using the following formula.

$$\% \text{ Inhibition} = \frac{100(\text{Control} - \text{Treatment})}{\text{Control}}$$

The concentration of the test compounds used to kill 50% of the of the growth of the cell lines, IC50 value was determined by using linear regression equation i.e. y=mx+c. Here, Y = 50, M and C values were derived from the viability graph.

2.4.RESULTS AND DISCUSSION:

In present work, eight formulations, F1 to F8 were tried to produce nanoparticles of Sorafenib tosylate by salting out method. This method is based on the separation of water miscible solvent from aqueous solution via salting out effect. The concentration of Zinc sulphate will prevent the miscibility of ethanol in to aqueous medium on mechanical agitation of this system, emulsion is formed. The formed emulation droplet size controlled by salting out agent. The increasing concentration of salting agent reduced the size of particles.

2.4.1.Drug content:

The results percent drug content of formulations are presented in **Table 2.** It was observed that as the drug to polymer concentration increases from F6to F8, Drug content were found to be acceptable with the range of 96.5% ±0.61 to 99.7%±0.55.

Table 2: percentage drug content of SRB nanoparticles

Formulation	% drug content (n=3±s.d)
F1	90.06±0.38
F2	90.77±0.38
F3	91.35±0.24
F4	92.15±0.13
F5	89.62±0.12
F6	88.04±0.30
F7	89.73±0.41
F8	89.33±1.02
F9	90.57±0.33

2.4.2.In-vitro drug release studies

Cellular uptake of anticancer drug delivery systems plays crucial role to elicit effective action against the cancerous tissues. Hence as an indirect

assessment the prepared formulations were assessed for in-vitro drug release studies. The results of drug releasing studies of F4 to F8 and pure Sorafenib tosylate are presented in **Table 3 and Fig. 1**. From the data it is observed that, there is enhanced % drug release from all the prepared formulations compared to pure drug release. Among all, formulation F3 evidenced high % drug release of 90.2% in 120 min and 82.5% in 90 min.

Table 3: Dissolution data of pure Sorafenib tosylate and prepared nanoparticles of sorafenib tosylate formulations, F4 to F8.

Time (min)	Pure drug	F1	F2	F3	F4	F5
10	7.38±0.35	19.6±0.18	26.9±0.25	35.5±0.21	10.35±0.51	8.35±0.21
20	8.43±0.18	26.5±0.35	31.4±0.34	49.9±0.12	18.7±0.42	14.5±0.63
30	9.05±0.36	32.8±0.24	45.3±0.16	58.7±0.35	19.5±0.42	18.4±0.11
45	10.71±0.24	39.6±0.26	51.2±0.17	65.9±0.58	23.9±0.35	22.7±0.43
60	12.83±0.11	43.6±0.15	66.4±0.27	76.4±0.35	26.4±0.15	24.3±0.31
90	15.10±0.58	51.9±0.14	69.4±0.22	82.5±0.39	29.6±0.89	27.2±0.62
120	17.5±0.42	61.5±0.19	70.4±0.37	90.2±0.22	47.6±0.21	42.5±0.11

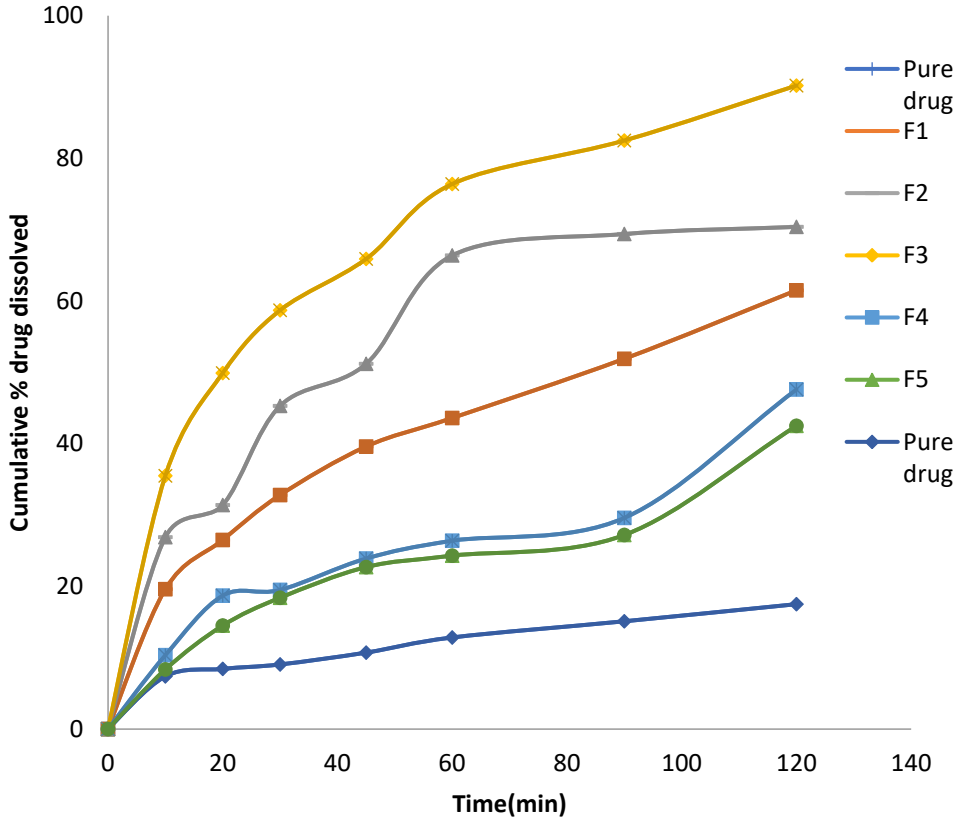


Fig.1: Dissolution curves of pure Sorafenib tosylate and prepared nanoparticles F1 to F5

Table 3: Dissolution data of pure Sorafenib tosylate and prepared nanoparticles formulations, F6 to F8.

Time	Pure drug	F6	F7	F8
10	7.38	11.1	18.2	20.8
20	8.43	20.2	25.3	31.1
30	9.05	25.3	26.7	35.5
45	10.71	30.4	36.3	47.2
60	12.83	40.4	42.8	53.2
90	15.1	47.5	49.9	54.7
120	17.5	52.8	52.2	59.3

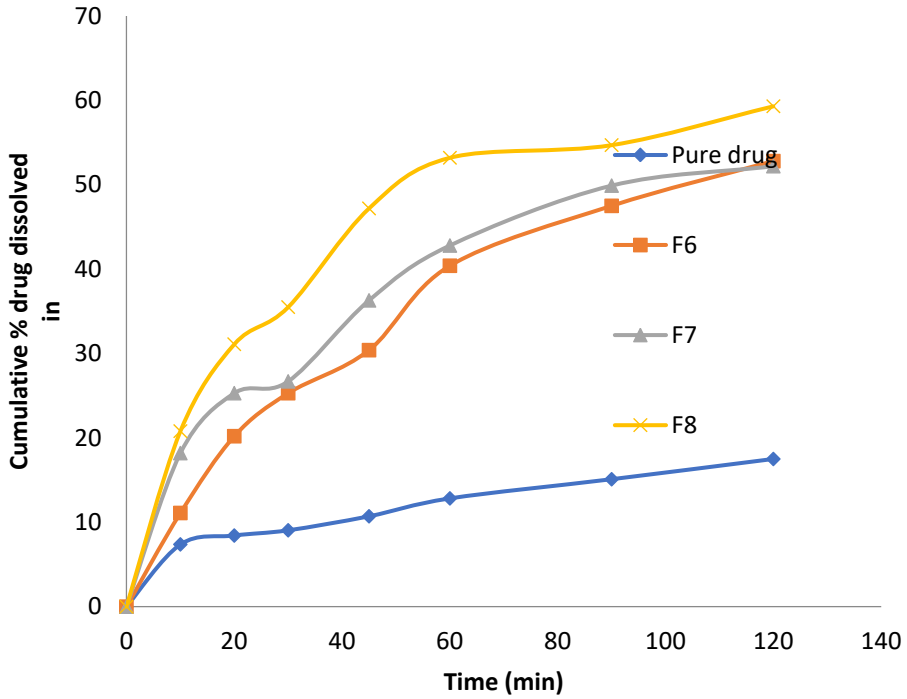


Fig.2: Dissolution curves of pure Sorafenib tosylate and prepared nanoparticles F6 to F8

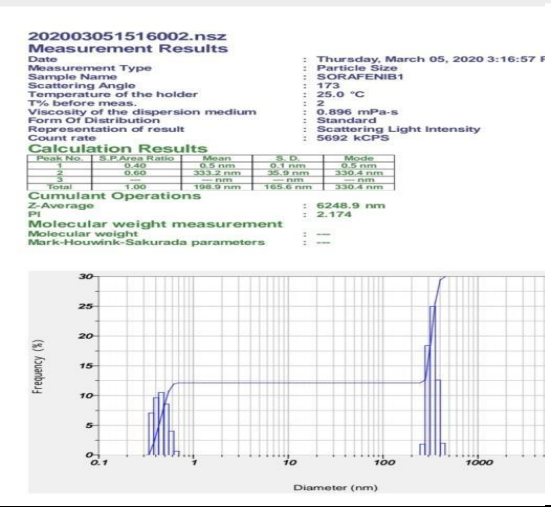
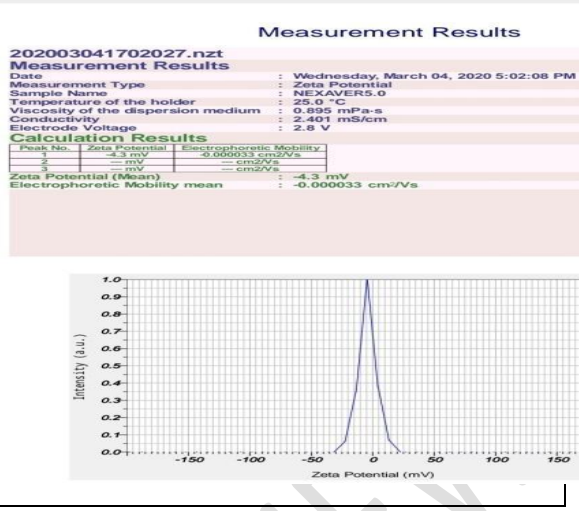
2.4.3.Particle size and zeta potential

The particle size of prepared nanoparticles of formulations F1 and F3 is determined as the drug release is high from them. The Scan copies indicating particle and zeta potential obtained from zeta sizer are presented in **fig. 3 and fig. 4** and the values are shown in **Table 4.** As shown in table and zeata sizer data. the mean particle size of formulation, F6 is 205.1nm and formulation F8 is 231.6nm. These results evidence that method used for preparing nanoparticles is successful in producing the yield in nano size range. Extremely negative values of zeta potential -4.3mv and -2.2mv indicates large repulsive forces showing the stability of prepared nanoparticles.

Table 4: Particle size and zeta potential determination

Formulation code	Particle size (nm)	Zeta Potential (my)
F1	198.8	-4.3
F3	231.6	-2.2

Fig.3:Particle size of Sorafinib	Fig.4:Zeta potential of nanoparticle

tosylate nanoparticles, F3	formulation, F3
	

2.4.5.Drug-excipient interaction studies by FT-IR:

The FI-IR spectra of formulation F2 and pure Sorafenib tosylate are given in **Fig.5 and 6** and the absorption peaks are shown in **Table 5.** Pure Sorafenib tosylate showed N-H stretch at 3134.7 **cm^{-1}**, C-H 17274 **cm^{-1}** at c=o stretch at 1699.13 **cm^{-1}** due to and C-N stretch at 1248.75 **cm^{-1}**. All these peaks are also present in spectrum of prepared nanoparticles formulation with slight change. Hence it is considered that there is no interaction between Sorafenib tosylate and the excipients used to prepare nano particles

Table 5. FTIR Spectra of Prepared Nanoparticles

Literature revealed	Absorption peaks in cm^{-1}	
	Pure Sorafenib tosylate	**Nanoparticles Formulation 1:3**
N-H	3134.7	3208
C-H	1727	1680
C=O	1699.13	1587
C-N	1248.75	1033

Fig.5: FTIR spectrum of pure drug Sorafenib Tosylate

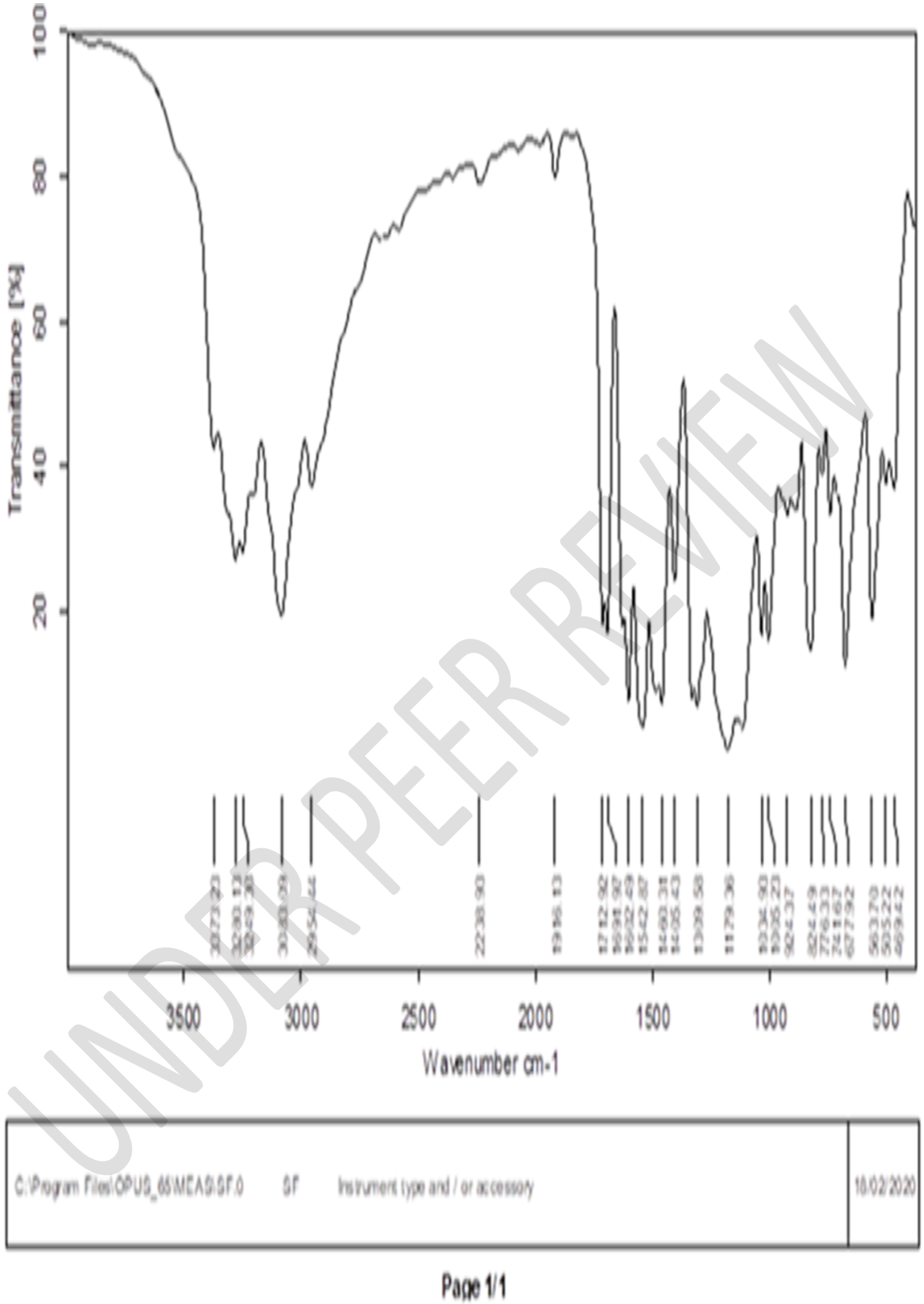
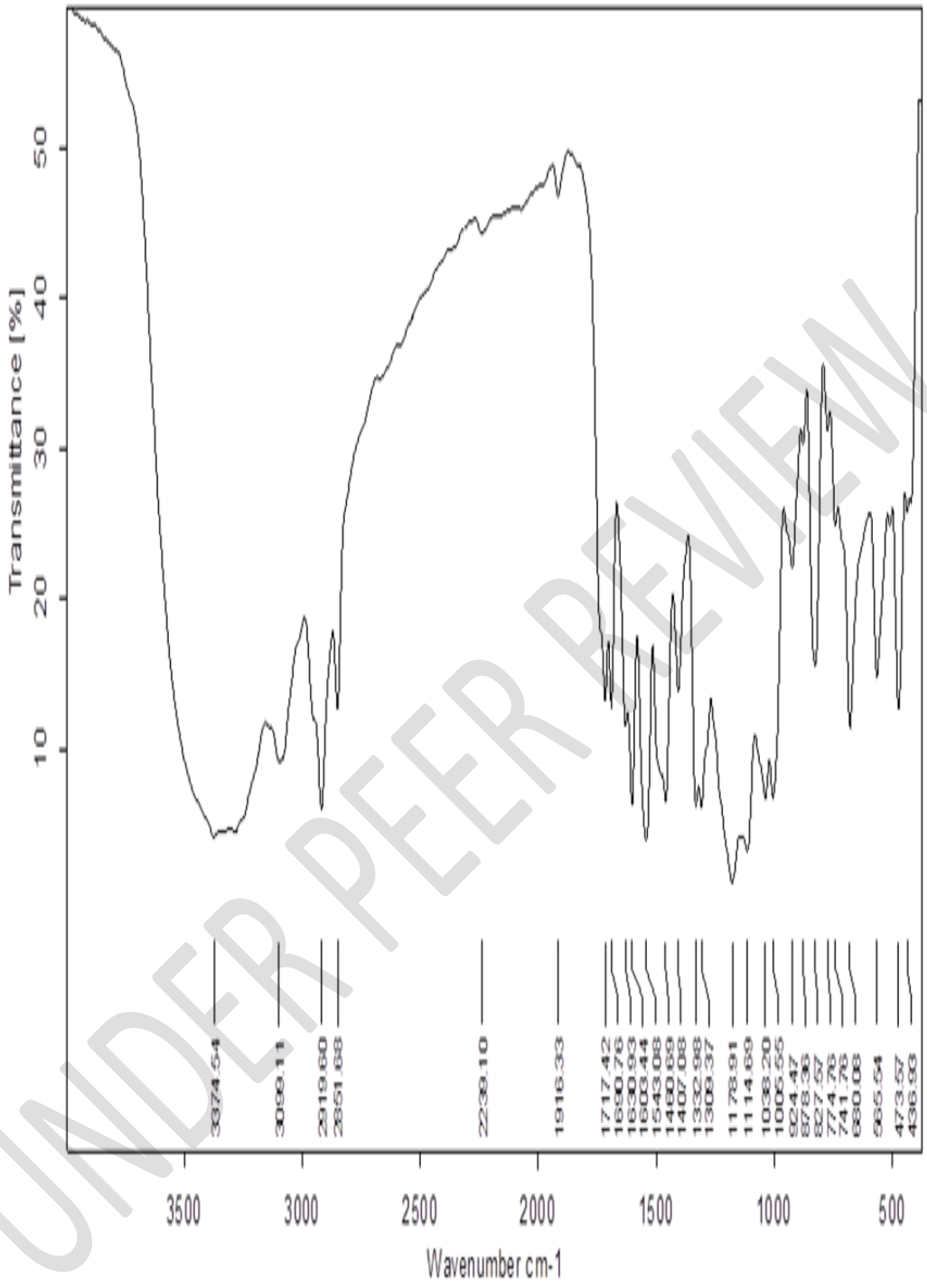


Fig.6:FT-IR spectrum of formulation Sorafenib Tosylate nanoparticle formulation F3



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2.4.6.Assessment of anticancer activity by MTT assay technique:

The results of evaluation of anti-cancer activity of promising nanoparticles of sorafenib tosylate as well as pure durgs are shown in **fig** 7 and respectively. The figures show much reduced staining in case of nanoparticles in **Fig.8** compared to pure drug in **Fig 7**. The data showing the % inhibition and % viability of cancer cells against the pure drugs and nano particles are shown in fig. 9-10 and tables 5-10. As presented, the data reveals IC50 value of Nano particles of 5-fluorouracil 0.518 ± 0.12 and pure drug is40.904 ± 0.0716**.** Similarly the IC50 value of Nano particles of sorafenib tosylate is 0.848 ± 0.217and pure drug is 1.92±0.140. Reasonable reduction in IC50 values shows the conversation of pure drug of 5 fluorouracil and sorafenib tosylate into nano particles are efficient in treatment of pancreatic cancer.

Table 6:Sorafenib nanoparticles:

Concentration (µg)	Absorbance at 570nm	% Inhibition	% Viability	IC_{50} (µg)
1	0.254	46.52	53.48	
2	0.176	62.94	37.06	
3	0.089	85.68	14.32	
4	0.053	88.84	11.16	
5	0.037	92.21	7.79	
Untreated	0.475	0	100	0.848±0.217
Blank	0	0	0	

Fig7:%viability vs. concentration of Sorafenib nanoparticles

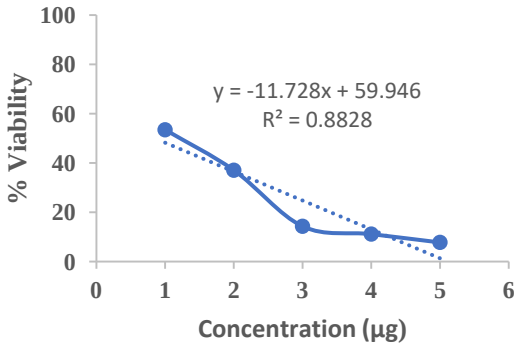


Table 7:Pure Sorafenib tosylate

Concentration	Absorbance	% inhibition	%viability	IC50=1.92
1	0.09	45	55	
2	0.15	51	49	
3	0.2	65	35	
4	0.31	75	25	
5	0.45	92	8	
Untreated	0.5	0	0	
Blank	0	0	0	

Fig8: %viability vs. concentration of Sorafenib nanoparticles

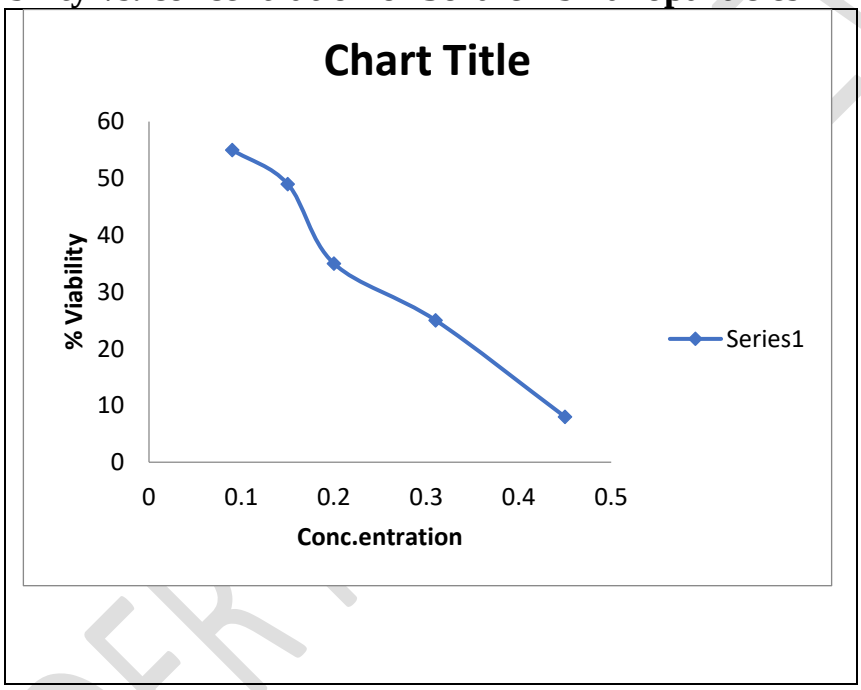
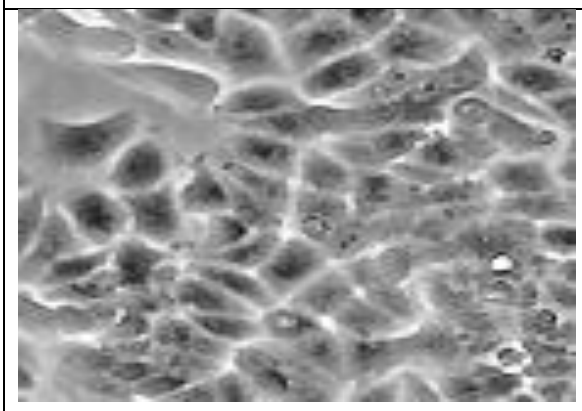
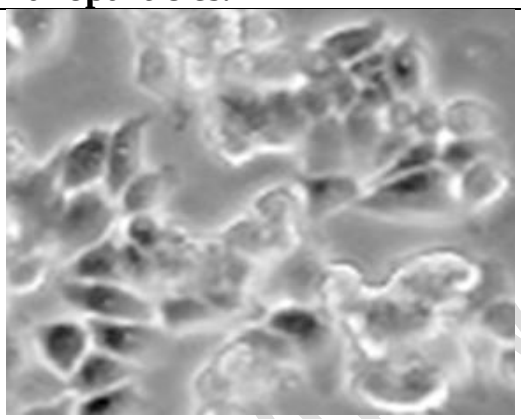


Table 8: IC 50 values of Test compounds

S. No	Sample Name	IC $_{50}$(µg)
1	Pure Sorafenib tosylate	1.92±0.14
2	Sorafenib tosylate nanoparticles	0.848 ± 0.217

Fig.9 Photograph PC Cells staining with Sorafenib tosylate	Fig. 10 Photograph PC Cells staining with Sorafenib tosylate nanoparticles.
	

2.5.CONCLUSION:

Sorafenib tosylate nanoparticles were successfully produced by salting out method using drug to polymer (Sorafenib tosylate: Eudragit L-100) ratio of 1:3 by salting out method to possess ideal drug release characteristics of 82.5% in 90min and 90.2% in 120min with average particle size 205.1nm. **IC50 values of nanoparticles of sorafenib tosylate are reasonably reduced compared to pure drugs indicating very chances of reduced side effects with nanoparticles produced by simple technique of salting out method and hence effective in treating pancreatic cancer.**

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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