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Research Article

Anticancer Nano Formulation of Imatinib with Chitosan Polymer

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ABSTRACT
Objective: Drug targeting is the capacity of the dosage form. In which the therapeutic agent acts specifically to desired site of action in the non-targeted tissue with the help of Nano particles is called as the drug targeting. IMATINIB is a used to treat cancer by chemo therapy. Cancers like chronic myeloid leukemia cancer (CML) and acute lymphoblastic leukemia cancer (ALL) and other specific types of gastrointestinal stromal cell tumor (GIST) systemic mast cell disease and Bone marrow failure disorder. It is administered by oral root. For ATP, Tyrosine kinase is act as a binding site. Methodology: The drug IMATINIB is loaded in the polymer chitosan, poly-(D) glucosamine is a bio compactible, bio degradable, nontoxic, antimicrobial and soluble in solvents. This preparation is done by emulsion-droplet coalescence method. Content of the Drug, Size of the particle and Zeta potential, Encapsulation efficiency and Drug release testing are described for this formulation in this study. Results: The Imatinib Nano particles were formulated and evaluated for its invitro drug release profile. Based on the invitro drug release profile of Imatinib nano particles formulation (INP1 – INP5) formulation INP3 was selected as the best formulation in which the particle size was 285.9nm. The invitro % drug release of INP3 formulation was 99.76 ± 0.82 and it was found to be the suitable formulation to manage the cancer. Conclusion: Hence it is concluded that the newly formulated controlled release nanoparticle drug delivery system of Imatinib may be idol and effective by allowing the drug to release continuously for 24 hrs.

Keywords: Imatinib –Anticancer nanoparticle, chitosan [poly (D) Glucosamine], Emulsion – Droplet Coalescence method.

INTRODUCTION

Imatinib, is a used to treat cancer by chemo therapy. Cancers like chronic myelogenous leukemia cancer (CML) and acute lymphocytic leukemia cancer (ALL)and certain other types of gastrointestinal stromal cell tumor (GIST) systemic macrocytosis and myelodysplastic syndrome. It is taken by oral root[1].

Chemical Structure

Molecular Formula

$C_{29}H_{31}N_7O$

Molecular Weight

493.603 g/mol

Iupac, Name:4-[(4-methylpiperazin-1-yl)methyl]-N-(4-methyl-3-{[4-(pyridin-3-yl)pyrimidin-2-yl]amino}phenyl)benzamide

CLASS: Chemotherapeutic agent

Commonly know brand name

Glivec 400 mg

Pharmacology and mode of action

Imatinib, for many tyrosine kinase enzymes - acts as a definite inhibitor. It is a 2-phenyl amino pyrimidine derivative. Which decreases the activity; it occupies the TK active site.

In human body there is numerous number of TK enzymes such as, the insulin receptor. Imatinib is specific for the TK domain in the Abelson proto-oncogene (abl), c-kit and platelet-derived growth factor receptor (PDGF-R).

In chronic myeloiod leukaemia, the fusion protein of Abelson proto-oncogene with breakpoint cluster region (bcr), termed bcr-abl has been led by the Philadelphia chromosomes. To decreases the bcr–abl activity Imatinib is used which is now a constitutively active tyrosine

Structure

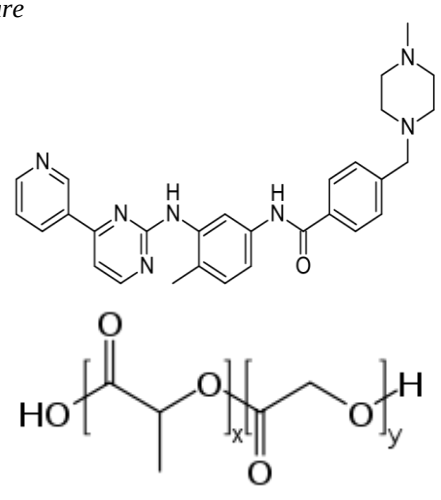

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Table 1: Materials used.

Materials	Supplier
Imatinib	Sigma aldrichpvt.ltd
Chitosan	Sigma Aldrich pvt.ltd
Poloxamer	Sigma Aldrich pvt.ltd
Ethanol	Sigma Aldrich pvt.ltd
Potassium di hydrogen phosphate	M/S SD Fine Chemicals, Mumbai, India
Ortho phosphoric acid	M/S SD Fine Chemicals, Mumbai, India

Table 2: Equipment list.

S.no	Equipments	Model
1.	Electronic balance	Mettler Toledo AG 135.
2.	Ultra-centrifuge	Remi instruments, Mumbai.
3.	Mechanical stirrer	Remi instrument.
4.	DSC	Shimadzu DSC-60.
5.	Particle size analyzer	Malvern master sizer.
6.	UV spectrophotometer	Shimadzu 1710, Mumbai.
7.	USP dissolution apparatus	Lab India, DS8000.

Table 3: Formula used for the formulation of Imatinib nanoparticles.

S.no	Formulation	Drug (mg)	Chitosan (%v/v)	Tween (%v/v)
1.	INP-1	100mg	0.5	5
2.	INP-2	100mg	1	5
3.	INP-3	100mg	1.5	5
4.	INP-4	100mg	2	5
5.	INP-5	100mg	2.5	5

kinase.

For ATP, a binding site is present in the active sites of tyrosine kinases have. The enzymatic activity catalysedfrom ATP, Protein Tyrosine Phosphorylation is the process in which a tyrosine kinase is the transfer of the terminal phosphate to tyrosine residues on its substrates. By locking it in a closed or self-inhibited conformation, Imatinib works by binding close to the ATP binding site of bcr-abl, and therefore the enzyme activity of the protein semi-competitively is inhibited.Many BCR-ABL mutations can cause resistance to imatinib by shifting its equilibrium toward the open or active conformation has been seen in this fact[2].

Pharmacokinetics

Imatinib when given through oral route it is rapidly absorbedand it is also highly bioavailable: In the bloodstream, 98% of an oral dose is reached. Liver plays an important role in the metabolism, several isozymes mediates it, such as -cytochrome P450 system, including CYP3A4 and to a lesser extent CYP1A2, CYP2D6, CYP2C9, and CYP2C19.the main metabolite T-demethylated piperazine derivative, it is also active derivative.They get mainly eliminated through bile andfecesand a small portion of the drug is excreted in the urine. Imatinib are eliminated in the form of metabolites; only one-fourth is eliminated unchanged form. The half-life of imatinib is 18hrs and its main metabolite is 40 hrs. The activity of Abelson cytoplasmic tyrosine kinase (ABL), c-Kit and the platelet-derived growth factor receptor (PDGFR) is inhibited. Imatinib mesylate appears to have utility in the treatment for the Indermatological diseases, due it's inhibition of PDGFR. Imatinib isefficient in the treatment such as FIP1L1-PDGFRalpha+ mast cell disease, hyper eosinophilic syndrome, and dermatofibrosarcoma protuberans[3].

Medical Uses[4]

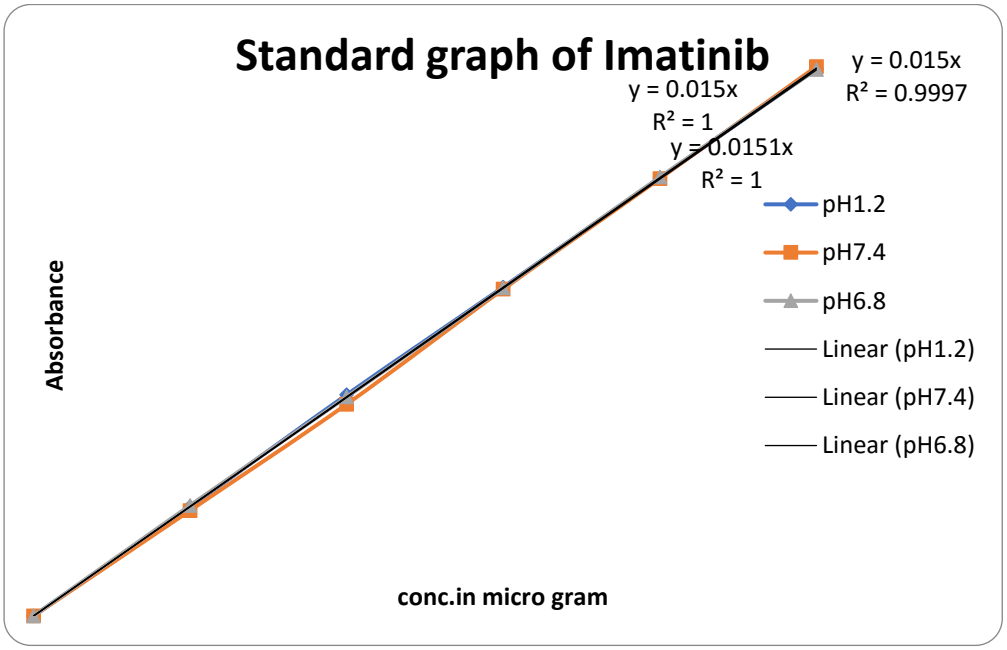


Figure 1: Calibration curve of Imatinib in pH 1.2, 7.4 and 6.8 buffers.

Table 4: Absorbance of Imatinib in buffer solutions.

S.No	Concentration	Absorbance		
		pH 1.2	pH 7.4	pH 6.8
1	10	0.150	0.145	0.152
2	20	0.304	0.291	0.301
3	30	0.452	0.449	0.451
4	40	0.602	0.601	0.603
5	50	0.752	0.755	0.751

Table 5: Physical characteristics for Imatinib.

S.no	Physical parameters	Results
1	Description	Off White powdered
2	Melting point	226°C
3	Loss on drying	0.11%
4	Assay	99.75%

Table 6: Physical characteristics for Individual drug and excipients.

S.no	Sample ID	Initial-description	Final-description
1	Imatinib	Off White powder	No change
2	Chitosan	Off-white powder	No changes

Table 7: Physical characteristics for drug-excipient mixture.

S.no	Sample ID	Initial-description	Final-description
1	Imatinib	Off White powder	No change
2	Imatinib+ Chitosan	Off White powdered	No changes

Table 8: Chemical characteristics for drug-excipient mixture.

S.no	Sample ID	Initial assay (%)	Final assay (%)
1	Imatinib	99.75%±0.13	99.72%±0.18
2	Imatinib+ Chitosan	99.76%±0.24	99.71%±0.16

Chronic myeloid leukemia (CML), Bone marrow failure disorder and a number of other malignancies are treated by the Drug Imatinib.

Contraindication

The hypersensitivityis the only known contraindication forthe imatinib.

Which includes:

Impairment of Hepatic function.

In patients with comorbidities, it may cause the risk of severe Heart impairment.

Risk of Teratogens during pregnancy.

Retentionof fluid is increased.

Stunted growth may occur in children or adolescents.

Profile

Chitosan- poly-(d)glucosamine

Characteristics5

Biocompatible, biodegradable, non-toxic, anti-microbial and soluble in wide range of solvents.

Solubility

Soluble in organic acids-acetic, adipic, lactic, succinic.

Phosphoric and sulphuric acid solution imatinib is insoluble

Chitosan should be first dissolved in aqueous solution, because it is insoluble in neutral or alkaline media.

Application

Flocculant, protein precipitation, encapsulating agent and aqueous thickener.

Properties

Molecular weight

1526.454g/mol

Inherent viscosity

200-800 Cp

MATERIALS AND METHODS

List of Materials

Methods

Preformulating studies:

Calibration graph for ImatinibPreparation

Preparation of calibration curve in pH 1.2, 7.4 and 6.8 buffer solutions

Imatinib was weighed accurately about100mgand in three, 100ml volumetric flask it was dissolved in small volume of buffer solutions. The volume was adjusted to 100ml with 1.2 pH buffer in the 1st Volumetric flask, the volume was adjusted to 100ml with7.4 pH buffer in the 2nd volumetric flask and the volume was adjusted to 100 ml with 6.8 pH buffer in the third volumetric flask. Imatinib with a concentration range from 10 to 50 µg/ml, were prepared for 1.2 pH buffer solution, 7.4 pH buffer solution and 6.8 pH buffer solution separately in the series of standard solution. At 285nm absorbance was measured and calibration graph was plotted using concentration versus absorbance.

Drug-excipient compatibility study by DSC (Differential scanning calorimetry)

Indirectly in pierced aluminum crucible pans,In Mettler Electronic balance thesamples of individual components along with each drug-excipient were weighed (5-10 mg) and under static airat the 50-300°C temperature range it is scanned, using shimadzu DSC-60 equipmentheating rate of 10 ºC/min.

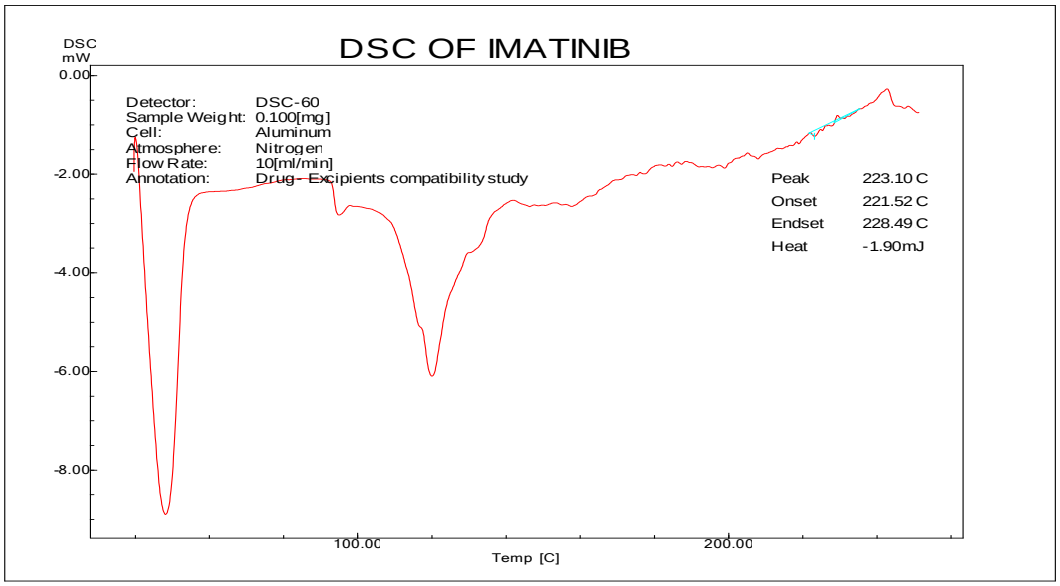
Method of preparation[6-9]

Method

Emulsion -droplet coalescence method

In 1% acetic acid, Chitosan was dissolved and 100 mg of Imatinib in phosphate buffered saline. In10 ml of liquid paraffin containing5% v/v tween 20, this mixture of chitosan and imatinib was added. Using a homogenizer this mixture was stirred for 3 min to make water in oil (w/o) emulsion.

At 3000 rpm the resultant Imatinib nanoparticles were centrifuged for 60 min and to remove the remaining surfactant and liquid paraffin, using ethanol and water the resultant is washed consecutively.



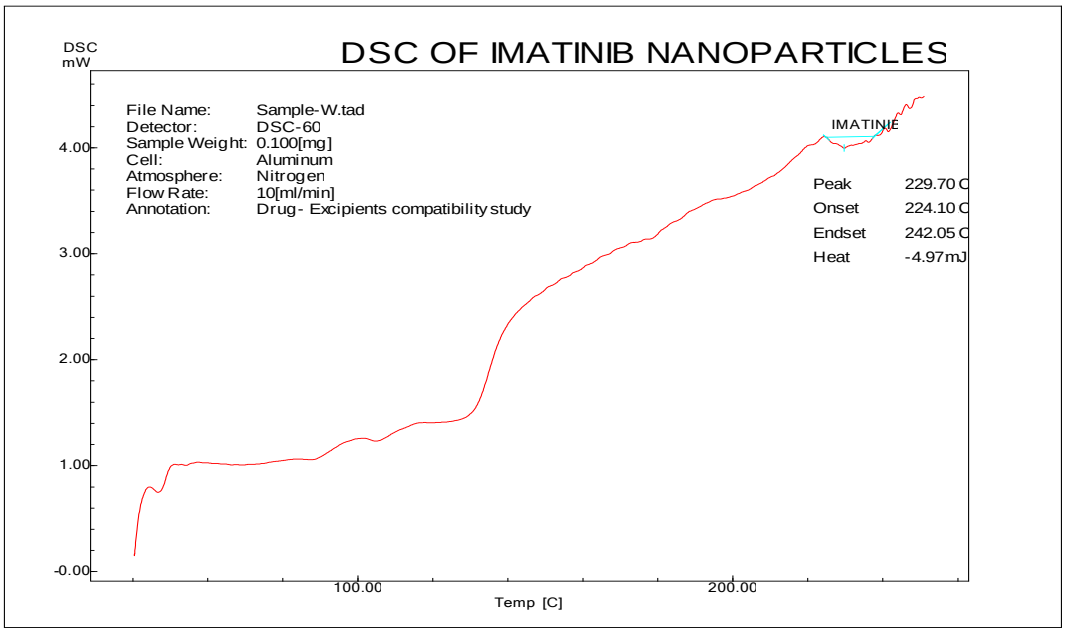


Figure 2 and 3: Preparation from this DSC study abd DSC thermogram.

Table 9: Drug content, Entrapment efficiency, Particle size and Zeta potential of Imatinib nanoparticles.

Trials	Zeta potential (mV)	Particle size(nm)	Entrapment Efficiency (%)	Drug Content (%)
INP1	17.8	326.8	70.81	99.71
INP2	16.3	300.2	72.65	99.74
INP3	10.6	285.9	85.47	99.78
INP4	9.5	276.2	85.64	99.73
INP5	8.9	270.4	85.83	99.72

For 3 hours, they were dried in air and at 50° by hot air oven for 4 hour and stored in a desiccator

Several batches namely (INP1, INP2, INP3, INP4, INP5)they were formulated by changing the drug and polymeric ratio and the effect of polymer concentration on the encapsulation efficiency and the drug loading capacity was performed.

Characterization Studies

Size of particle and zeta potential

Content of the drug

Encapsulation efficiency

Drug release testing.

Particle size and Surface charge

In adhesion and interaction of particle with cells, surface charge was important. Measure the cell surface charge density, the zeta- potential is used. By usingMalvern-Zeta sizer. Using zeta sizer, the prepared nanoparticles are evaluated for their particle size and surface charge by photon correlation spectroscopy (PCS).To get a suitable kilo counts per second (kcps), the formulations were diluted to 1:1000 with the aqueous phase of the formulation. At 25°C with an angle of detection of 90°

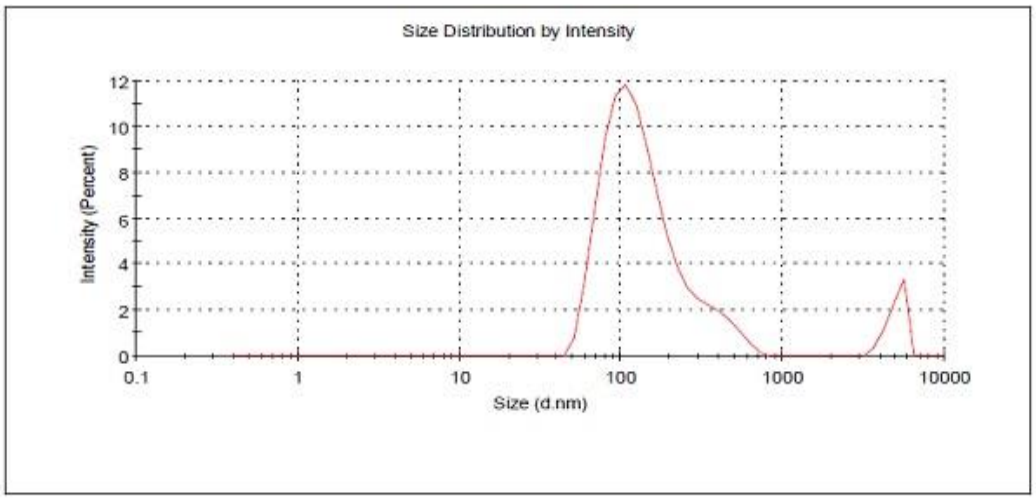


Figure 4: Particle size of optimized Imatinib loaded Chitosan nanoparticles (INP3).

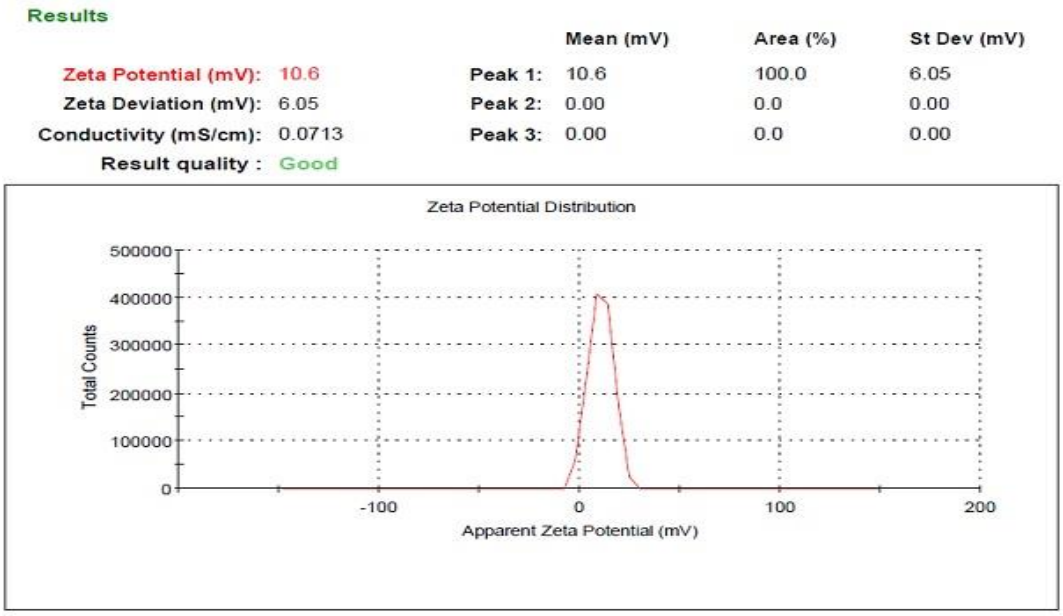


Figure 5: Zeta potential of optimized Imatinib loaded Chitosan nanoparticles (INP3).

Table 10: *In vitro* release studies of Imatinib nanoparticles.

S.No	Time (Hrs)	%Cumulative drug release				
		INP1	INP2	INP3	INP4	INP5
1	0.5	45.78± 0.12	40.97± 0.25	25.86± 0.29	15.83± 0.34	12.62± 0.13
2	1	52.86± 0.35	49.83± 0.71	35.91± 0.16	30.72± 0.18	24.78± 0.87
3	6	61.97± 0.56	56.78± 0.28	50.73± 0.23	41.34± 0.45	30.25± 0.46
4	12	99.76± 0.67	76.92± 0.16	65.82± 0.17	58.83± 0.19	40.43± 0.43
5	16	99.73± 0.44	90.82± 0.34	73.93± 0.41	69.76± 0.22	53.23± 0.15
6	20	99.74± 0.18	99.74± 0.27	88.45± 0.29	80.43± 0.56	65.58± 0.34
7	24	99.72± 0.32	99.73± 0.19	99.76± 0.82	92.81± 0.65	73.82± 0.26

mean±S.D, n=3

analysis were carried out. In this experiment six replicates were taken for the measurement. The result was given in results and discussion section.

Drug content

Accurately weighed 1gm of Imatinib nanoparticles was weighed and transferred into 25ml standard volumetric flask and then the sample was dissolved with 0.1M HCl. 1ml of the solution and diluted with the same buffer solution to 25ml. The regular Imatinib was dissolved and diluted with same buffer solution.

Using UV-Visible spectrophotometer, at 285nm the standard and sample absorbance was measured. The percentage of drug content was calculated[10].

Entrapment efficiency

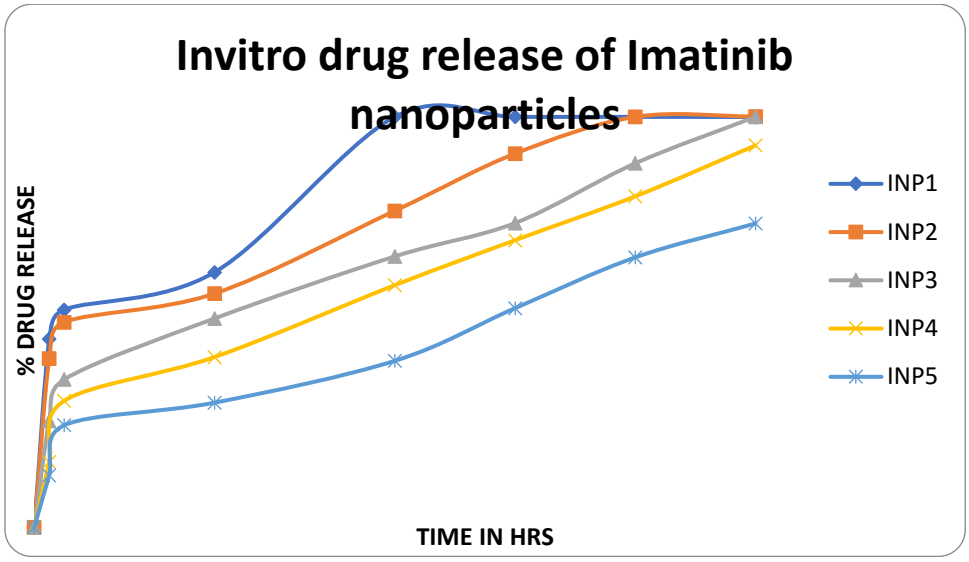


Figure 6

The Nanoparticles loaded with drug was centrifuged at 15000 rpm for about 30 mins.1 ml of the separated supernatant liquid was diluted and was measured for the absorbance at about 285 nm The Imatinib amount unentrapped in the supernatant was calculated by subtracting quantity of unentrapped Imatinib from the total amount of Imatinib used for the preparation, the amount of Imatinib entrapped was determined.

To calculate entrapment efficiency the formula used was given below

$$\text{Drug entrapment}(\%) = \frac{\text{mass of drug in nanoparticles x100}}{\text{mass of drug used in formulation}}$$

In vitro release

Franz diffusion cell is used to study the invitro release by means of a dialysis membrane. Inside a dialysis membrane, the prepared Imatinib nanoparticles formulations were placed and immersed in 0.1M HCl. product is keptaside and evaluated to check the released quantity of Imatinib at predetermined time intervals & thus absorbance is calculated at 285 nm by means spectrophotometer and result was calculated based on the values obtained by the absorbance and noted in the results and discussion part[11].

RESULT AND DISCUSSION

Preformulating studies

calibration graph for the Preparation of Imatinib

Imatinib,Standard calibration data in pH ranging 1.2, 7.4 and 6.8 buffers at 285 nm

Standard calibration curve of Imatinib was carried out in 1.2 pH, 7.4 pH and 6.8 pH buffer at 220 nm. The r^2 value in the entire medium shows nearly 1, which signifies linearity.

DSC analysis

DSC of Imatinib showedat about 223^0C (melting point) a sharp endothermic peak. The physical mixture of Imatinib with other excipients gives the similar value (229^0C) and DSC

outcome for mixture of Imatinib and other excipients also have the parallel analogue in the thermogram and there was no significant change observed.

It was found there is no specific relation for Imatinib and other excipients added in this

Accelerated compatibility study of Drug –Excipients - Physical observation and assay

No colour change was observed in the drug excipient mixture and based on the chemical evaluation there is no significant changes indicates that the drug is compatible with the ingredients added and these results were given in the Table no. 3.2

With increasing Chitosan concentration, theparticle size and entrapment efficiency of the Imatinib nanoparticles (INP1- INP3) were increased

Increasing the Chitosan concentration due to drug coated layer is increased, thus it increased particle size and entrapment efficiencyis as a result oflarge quantity of availability of Chitosan to encapsulate the drug.

Because of drug availability to be integrated is low which is not enough for further encapsulation of drug by Chitosan, the Chitosan concentration (INP4-INP5) is increased further and there is no much increase in the entrapment efficiency.

In- vitro drug release

Effect of Chitosan concentration on Invitro drug release of Imatinib nanoparticles

From the results of *In vitro* drug releasestudythe end of 24h, the maximum percentage drug release (99.76± 0.82) at was observed with trial INP3that contains 1.5% w/v of Chitosan.

Below 1.5% w/v of Chitosan concentration as in the case of trials INP1 and INP 2 the maximum percentage drug release 99.72± 0.32and 99.73± 0.19 were obtained at the end of 12 and 20 respectively which was not desirable.

Above 1.5% w/v of Chitosan concentration, reduction in drug release was observed as in the case of trial INP4 and INP5. The maximum percentage drug release for INP4 and INP5 were found to be 92.81± 0.65 and 73.82± 0.26respectively at the end of 24h was obtained.

From the result of*In vitro* drug releasefor INP1- INP5, it was found that prepared nanoparticles,there is increase in concentration of Chitosan slows down the release of drug

as a result of increase in the size of particle and reduction in surface area.

Among the 5 different formulations, due to its ideal particle size (285.9nm), high entrapment efficiency (85.47%) and desirable drug release (99.76± 0.82% at the end of 24 h), INP3 was selected as effective formulation.

SUMMARY AND CONCLUSIONS

The active pharmaceutical ingredient Imatinib was evaluated for its Organoleptic properties and solubility. The results obtained were satisfactory.

Imatinib nanoparticles was prepared by emulsion droplet coalescence method and the polymer concentrations were optimized by various trials

In the present study Chitosan nanoparticles containing Imatinib was prepared. The effect of increase in Chitosan concentration in various parameters like particle size and *invitro* release profile were studied.

The Imatinib nanoparticles were formulated and evaluated for its *invitro* drug release profile. The results showed that the in vitro drug release for INP1, INP2, INP3, INP4 and INP5 were found to be 99.72± 0.32, 99.73± 0.19, 99.76± 0.82, 92.81± 0.65 and 73.82± 0.26 respectively.

Based on the result of *In vitro* drug release study, Imatinib Nanoparticles Formulations (INP1-INP5) formulation INP3 was selected as the effective formulation in which the particle size was 285.9nm.

The result of *In vitro* % drug release of INP3 formulation was 99.76± 0.82 and it was found to be suitable formulation to manage the condition of cancer. Hence it is reported that newly formulated nanodrug practical controls the release nanoparticulate drug deliverance of Imatinib may be ideal and effective by allowing the drug to release continuously for 24 hrs.

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