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THE DESIGN AND BIOPHARMACEUTICAL EVALUATION OF NANOPARTICLES CONTAINING 5-FLUOROURACIL (5-FLUORO-H,3-H-PRIMIDINE2,4-DIONE)

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ABSTRACT

Cancer is a major public health concern in both developed and developing countries. Now a days, the treatments of cancer categorize in to 5 classes, Surgery, Radiation, Chemotherapy, targeted and immunotherapy. Cancer chemotherapy is one the major way to treat cancer without medical assistants. The main aim of the ideal cancer chemotherapy is to deliver the correct amount of drug with desired controlled rate and sufficiently longer duration of time to the site of action (Tumor cells) to obtain the desired therapeutic response. Traditional cancer chemotherapy is based on the promise that tumor cells are more likely to be killed by anticancer drugs because of the faster proliferation of those cancer cells. However, in reality most of the drugs cannot differentiate cancer cells from the normal cells. This results in the undesirable effect of the drug because of the lack of selectivity. To minimize this risk, the development of tumor targeted drug delivery systems of anti-cancerous agents is necessary. The success of therapy depends on the selection of ideal carriers' system that can deliver the drug selectively to tumor cells. These carries include nanoparticles, nanotubes, nanorods, dendrimers, micelles, solid lipid nanoparticles, microspheres.

KEYWORDS: *Nanaoparticle, Nanomedicine, polythyleneimine, 5-Fluorouracil, Biotin etc.*

INTRODUCTION

WHO reported that Cancer is one of the leading causes of morbidity and mortality world -wide with 14 million cases approximately in 2012.According to the Union for international Cancer control (UICC), over 7 million people die of Cancer, and more than 11 million new cases are diagnosed world-wide. In next two decades, it is expected to be raise the number of new cases to about 70%, It was reported that cancer is responsible for 8.8 million deaths in 2015.Cancer is a major public health concern in both developed and developing countries. Now a days, the treatments of cancer categorize in to 5 classes, Surgery, Radiation, Chemotherapy, targeted and immunotherapy. Cancer chemotherapy is one the major way to treat cancer. The aim of the ideal cancer chemotherapy is to deliver the correct amount of drug with desired controlled rate and for sufficiently longer duration of time to the site of action (Tumor) cells, while prevent the normal cells to obtain the desired therapeutic response.

A nanoparticle is a particle having the size range of 1-1000 nm in dimension. The 5-Flurouraci l (5-Fluoro-1 H,3H-pyrimidine-2,4-dione) is a known earlier antineoplastic, antimetabolite. All the materials used in the experiments were of analytical grade. The optimization of individual material was done the 5-FU was found that the in-vivo drug release of 5-FU from BPEI-PLGA NPs at pH 7.4 and pH 5 was best explained by Higuchi's Model, as the plot showed the highest linearity with the regression coefficient of 0.989 and 0.990 respectively. It was found that the in-vivo drug release of 5-FU from BPEI-PLGA NPs at pH 7.4 and pH5 was best explained by Higuchi's Model, as the plot showed the highest linearity with the regression coefficient of 0.989 and 0.990 respectively.

5-FLUOROURACIL (5-FLUORO-H,3H-PYRIMIDINE-2,4-DIONE)

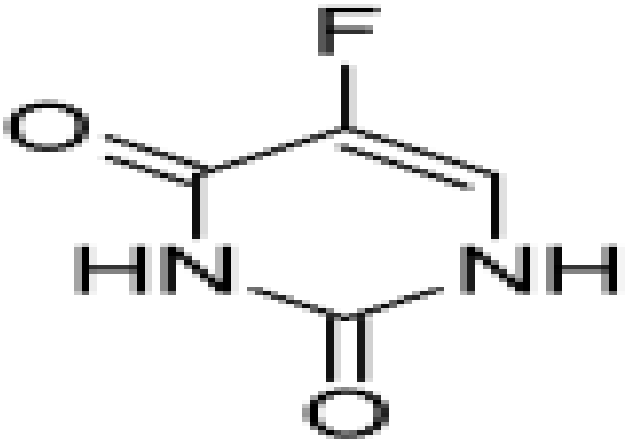


Figure: Chemical structure of 5-Fluorouracil.

Description

Empirical formula: C4H3FN2O2, **CAS number** is 51-21-8, **Molecular weight** 130. 077223 g/mol, IUPAC **name**: 5-Fluoro-1 H,3H-pyrimidine-2,4- Dione **Category:** Anti-neoplastic, antimetabolite, Pyrimidine analogu5-Fluorouracil is a white to nearly white crystalline powder, practically odorless.

Melting point: **282 - 283º C**

Solubility: 1 gm in 80 ml;170 ml Ethyl alcohol & 55 ml methanol; practically insoluble in chloroform, ether & benzene; solubility in aqueous increases with increase pH of solution.

Mechanism of action

5-FU requires enzymatic conversion to the nucleotide status (ribosylation and phosphorylation) to exert its cytotoxic activity. Several routes are available for the formation of floxuridine monophosphate (FUMP). Uridine phosphorylase converted 5-FU to fluorouridine and then to Floxurine monophosphate by uridine kinase, or it may be reacted directly with 5-phosphoribosyl- 1 – pyrophosphate (PRPP), catalyzed by orotate phosphor ribosyl transferase, to form FUMP.

Further, metabolic pathways are available to FUMP as the triphosphate FUTP, it is incorporated in to RNA. In an alternative reaction sequence crucial for antineoplastic activity, it is reduced to FUDP by ribonucleotide reductase (RNR) to the deoxynucleotide level and forming of Dump. 5-FU may also be converted by thymidine phosphorylase to the deoxy riboside fluor-deoxy uridine (Fur) and then by thymidine kinase to FdUMP, a potent inhibitor of thymidylate synthetase (TS), FdUMP inhibits TS and blocks the synthesis of TTP, a necessary constituent of DNA. The folate co-

factor Methylene-tetrahydrofolate and FdUMP form a covalently bound ternary complex with TS. This inhibited complex resembles the transition state formed during the enzymatic conversion of dUMP to thymidylate. The physiological complex of TS folate-dump progresses to the synthesis of thymidylate by transfer of the methylene group and two hydrogen atoms from folate to dUMP, but this reaction is blocked in the inhibited complex of TS-FdUMP-folate by the stability of the fluorine carbon on FdUMP; sustained inhibition of the enzyme results.

Pharmacokinetics

Intravenous administration of 5-FU produces peak plasma concentration of 0.1-0.5 µM, plasma clearance is rapid (with a $t_{1/2\ of}$ 10-20 minutes). Only 5-10% of a single intravenous dose of 5-FU is excreted intact in the urine. Given by continuous intra venous infusion for 24-120 hours,5-FU achieves steady state concentrations range of 0.5-0.8 µM.5-FU enters the CSF in minimal ammo.

PLGA

PLGA is approved by FDA and European Medicine Agency in drug delivery systems for parenteral administrations. PLGA is a synthetic copolymer of lactic acid (α –hydroxyl propionic acid) and glycolic acid (α-hydroxy acetic acid). Lactic acid contains an asymmetric carbon and therefore has 2-optical isomers. They are (+) lactic acid and D (-) lactic acid. Lactic acid contains in all living organism and is the intermediate or end product of carbohydrate metabolism. In vivo degradation of PLGA leads to the formation of harmless products. Final degradation products are lactate (salt of lactic acid) and glycolate (salt form of lactic acid).

Polyethyleneimine as cat-ionic polymer

PEI is a polymer that has been used for common processes such as paper production, shampoo manufacturing and water purification. Two types of polymers are available: Linear and branched. The branched type is formed by cationic polymerization of Aziridine monomers, via chain growth mechanism for gene therapy. The branched form of PEI contains 1º,2º and 3ºamines, each with a potential to be protonated. This gives PEI the attribute of serving as an effective buffer through a wide pH range. With nitrogen appearing as one out of every three atoms in the PEI backbone, any benefits of branching and proton-ability quickly accumulate in relation to the overall polymer size.

Use of Biotin as targeting ligand: -Biotin is a water-soluble vitamin generally classified with the B-complex also known as vitamin B7 or coenzyme R. Biotin is composed of a ureido (tetra-hydroimidazolone) ring fused with a tetrahydrothiophene ring. At the cellular level biotin act a growth promoter and works as a coenzyme for carboxylase enzymes in the preparation of fatty acids, isoleucine and valine and participates in gluconeogenesis. Biotin plays a key role in cell signaling, epigenetic gene regulation and chromatin structure.

PREPARATION OF PLGA nanoparticles

Nanoparticles (NPs) were prepared by double emulsification(W1/O/W2) solvent evaporation technique. It involves 2 major steps,

1. The formation of stable droplets of the primary emulsion and the removal of solvent from the droplets of secondary emulsion. 50 mg of PLGA was dissolved in a mixture of Dichloromethane(2ml) and acetone (2ml). Then add ultrapure water (1 ml) to the PLGA solution followed by ice water sonication for 2 minutes to form primary emulsion(W1/O).

2. The primary emulsion was added drop-wise to 0.5% PVA solution(20ml) under stirring at 1500 rpm. White color secondary emulsion(W2/O) was formed. Thus, formed emulsion was sonicated for 30 min. the emulsion was mechanically stirred for overnight to allow the evaporation of organic solvent. Filter the NP suspension through cellulose nitrate membrane0.8 µm, The NPs were collected by centrifugation at 6000 rpm for 15 minutes. Washing thrice with ultrapure water and then freeze dried to obtain NPs as dry powder.

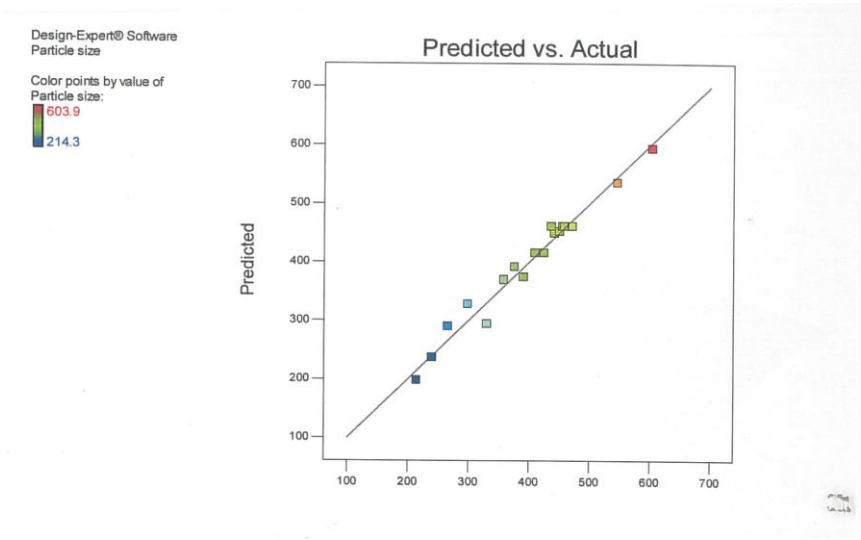
OPTIMIZATION OF PREPARATIONOF PLGA NPs

Optimization of nanoparticles was carried with central composite statistical design with 3 factors, 5 levels and 20 runs were selected for the study design using Design Expert 10.0.2 software trial version (state –Ease inc, Minneapolis, (USA). This design is suitable for exploring quadratic response surfaces and constructing second order polynomial models.

BC	8109.01	1	8109.01	16.45	0.0023	
A^2	43431.52	1	43431.52	88.99	<0.0001	
B^2	24859.32	1	24859.32	50.42	<0.0001	
C^2	3452.26	1	3452.26	7.00	0.0245	
Residual	4930.26	10	493.03			
Lack of Fit	4034.58	5	806.92	4.50	0.0621	Not significant
Pure Error	895.68	5	179.14			
Cor Total	1.856E+005	19				

The Model F-value of 40.71 implies that the model is significant. The “Pred – R-Squared of 0.8228 is in reasonable agreement with the “ADJ R-squared” of 0.9495; i.e, the difference is less than 0.2, Adeq Precision” measure the signal to noise ratio. A ratio greater than4 is desirable. Ratio of 25.199 indicates an adequate signal. This model can be used to navigate the design space.

ANOVA graph of predicted value v/s Actual value



This model proposed the following the following polynomial equation in terms of coded factors for particle size of PLGA NPs.

$$R1 = 461 + 40.87A + 29.00B + 53.13C + 42.81AB + - 41.91AC + 31.84BC + -54.90\ A^2 + -41.53\ B^{2\ +} 15.48\ C^2$$

Where R1is the particle size, A is the concentration of PVA solution is the sonication time and C is the Volume of outer Phase.

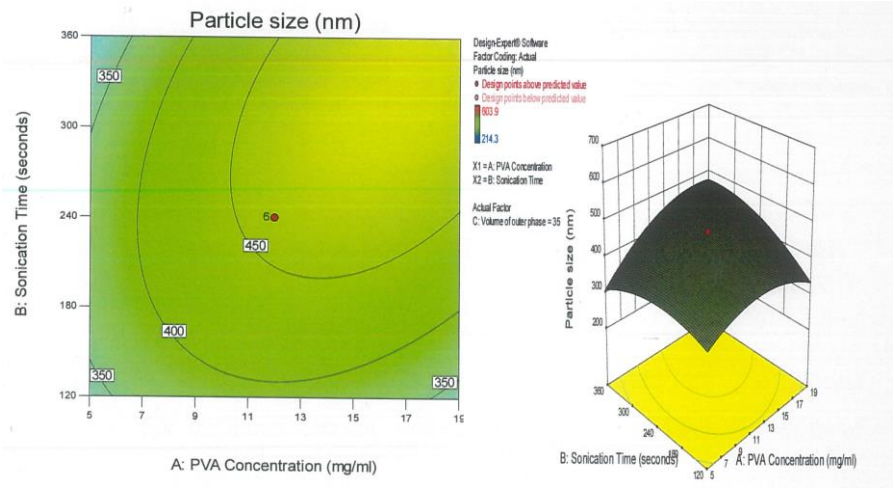


Figure: Contour plot of particle size of PVA solution.

Optimized formula

The Optimized formula was determined after studying the effect to find dependent variables on responses. The criteria followed for generating the optimized formula based on selecting the individual Variable and defining their goal and limits. The table gives the optimization Constraints selected for reaction variable. After defining constraints for each variable, Design Experts software ®automatically generated the optimized formula. In Table gives the optimized Formula generated by Design Expert software® showing the predicted and the experimental Values obtained. It was observed that the percentage prediction error was low (3.51%) which indicates the accuracy of the prediction by the software and the utility of the experimental design for process optimization.

Optimization Constraints selected for optimization of PLGA NPs

Variables	**Constraints**		
Independent variables	**Lower limit**	**Upper limit**	**Goal**
A =Concentration of PVA solution	5	19	In range
B = Sonication time	120	360	In range
C = Volume of outer phase	15	55	In range
Dependent variables			
R1 = particle size	214.3	603.9	minimize

Table: Predicted and experimental values obtained based on optimized formula for preparation of PLGA NPs.

Predicted values based on Optimized formula			
Factors			Responses
Concentration of PVA solution(mg/ml)	Sonication time (seconds)	Volume of outer phase(ml)	Particle size (nm)
5.00894	348.988	15.0182	210.3
Experimental values based on optimized formula			
Factors			
Concentration of PVA solution(mg/ml)	Sonication time (seconds)	Volume of outer phase(ml)	Particle size (nm)
5.00894	348.988	15.0182	217.7
Percentage Prediction error			3.51

DRUG LOADING

Drug loading was done with the optimized formula Hydrophilic drug was incorporated by dissolving the drug in aqueous phase. For the drug loading dissolve 5-FU in ultra-pure water (10 mg/ml).50 mg PLGA was dissolved in a mixture of dichloromethane (2 ml) and acetone (2 ml). Then add 5-FU aqueous solution (1 ml) to the PLGA solution followed ice water, sonication for 348 seconds to form primary emulsion. The primary emulsion was added drop wise to 5mg/ml PVA solution (15 ml) under stirring at 1500 rpm. White color secondary emulsion was formed. Thus, formed emulsion was sonicated for 30 min and the emulsion was mechanically stirred for overnight to allow the evaporation of organic solvent. Filter the NP suspension through cellulose nitrate membrane 0. 8µm.The NPs were collected by centrifugation at 6000 rpm for 15 minutes. Washing thrice with ultrapure water and then freeze dried to obtain NPs as dry powder.

SYNTHESIS OF BIOTINYLATED POLYETHYLENEIMINE (BPEI)

Biotin was conjugated to branched polyethyleneimine for the preparation of BPEI. Biotin (30 mg) was activated with NHS (14 mg) and EDC (23mg) in dimethyl sulfoxide (3ml). The activated biotin solution was added to 25KDa PEI (200mg) in de-ionized water (12 ml). The reaction mixture was stirred at 20 ^{0} C for 24 hrs. As formed biotinylatepolyethylineimine was purified by dialyzed against water for 2 days (MWCO:8000 -12000). Purified product was lyophilized and defined as biotinylated polyethyleneimine (BPEI).

PREPARATION OF BIOTINYLATED POLYETHYLENEIMINE MODIFIEDPLGA NANOPARTICLES (BPEI-PLGA NPs) PLGA NPs surface was modified with biotinylated polyethyleneimine through EDC, NHS reaction.100 mg of freeze-dried nanoparticles was dispersed in ultrapure water and add EDC (40mg) and NHS (20mg) for the activation of carboxyl group on the nanoparticle surface. After 4 hours add synthesized BPEI (60mg) to the activated PLGA NPs suspension, Stirred 24 hours. After 24-hour BPEI-PLGA NPS was centrifuged at 6000 rpm for 20 minutes, collected the nanoparticles and discard excess coupling reagent and un-conjugated BPEI in supernatant solution. Washed the BPEI-PLGA NPS thrice, freeze dried the product by using mannitol as cryoprotectant. Freeze dried BPEI-PLGA NPS was stored under 2-8 ºC.

Figure: Drug loaded loaded PLGA NPs.

CHARACTERIZATION OF BIOTINYLATED POLYETHYLENEIMINE MODIFIED NANOPARTICLES

FTIR spectroscopy: -FTIR spectrum of BPEI-PLGA NPs was obtained and the spectrum shows characteristic peaks at 1759 cm-1 due to C- O stretch of PLGA, peak s at 2928 cm-1 and 2854 cm-1 due to asymmetric and symmetric

stretching of CH groups. Peak at 680 cm -1 due to C-S stretching of biotin. NH stretching bands formed in between 3100-3400 cm-1. Presence of characteristic peaks belongs to BPEI confirmed the surface modification on PLGA NPs.

Mean particle size: - The mean diameter of BPEI-PLGA NPs was found to be 275.8 nm. Surface modification with BPEI increase the mean diameter by 58nm.

Mean zeta potential

Surface modification was clearly confirmed with zeta potential of BPEI –PLGA NPs. Zeta potential was found to be 24.1. Unmodified drug loaded PLGA NPs zeta potential was found to be -22.8. So, the surface modification with BPEI reverse the surface charge from negative to positive zeta potential. In theory the more pronounced zeta potentials in the range of 20 -40 mV, being positive or negative, is favorable for stabilizing the nanoparticles in colloid solution. BPEI – PLGA NPs have zeta potential of 24 mV, so it stabilized the nanoparticles.

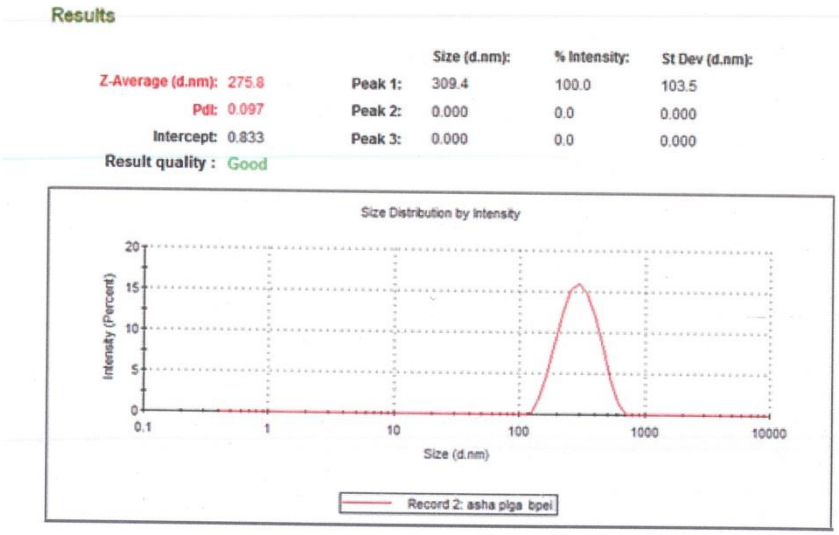
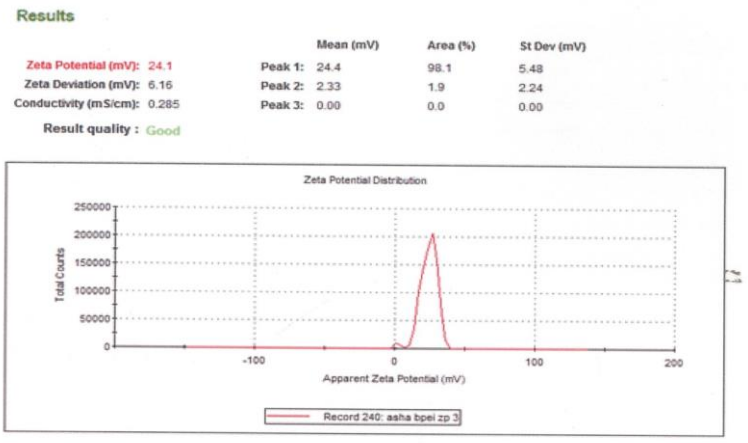


Figure: Mean particle size of BPEI-PLGA NPs.



Mean Zeta potential of BPEI-PLGA-NPs

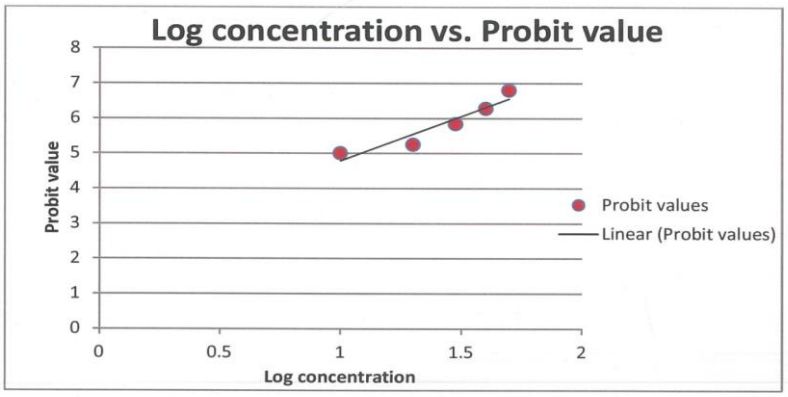
Transmission Electron Microscopy: - Surface modification with BPEI maintained the spherical shape of PLGA NPs. The low-density region in the image indicated the surface modification with BPEI. The BPEI-PLGA NPs was almost

non disperse in nature. The particle size ranges obtained from dynamic light scattering were in consonance with that of TEM.

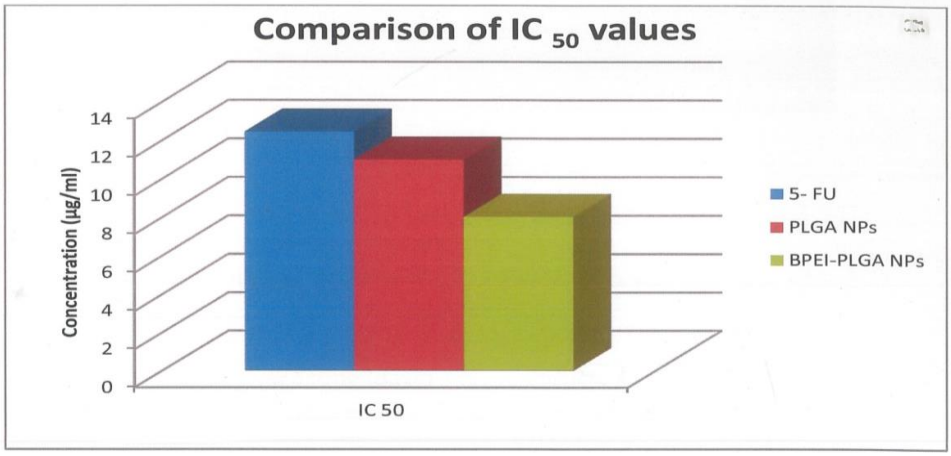
Determination of Encapsulation efficiency: - Encapsulation efficiency of 5-FU loaded BPEI_PLGA NPs was found to be 61%, Encapsulation efficiency was reduced from 65% to 61% due to small quantity of 5-FU leaked during surface modifications. However, the 5-FU leak might be favor to reduce the drug distribution on the surface layer of nanoparticles.

In –vitro cytotoxic assay

The in-vitro toxicity assessment of the prepared nanoparticles and free 5-FU was done on HeLa cell line and the data was tabulated below



Plot of log concentration vs. probity value of BPEI-PLGA_NPs



Comparison of IC50 of free 5-FU and nanoparticles

Table: MTT assay of Free 5-FU.

Concentration of 5-FU (µg/ml)	Average optical Density of control at 540 nm	Average optical Density of test at 540 nm	Percentage validity
1.25	0.798±0.0130	0.697	87.46
2.5		0.621	77.81
5		0.533	66.79
10		0.403	50.50
20		0.261	32.7

MTT assay of BPEI-PLGA NPs

Concentration of 5-FU (µg/ml)	Average optical Density of control at 540 nm	Average optical Density of test at 540 nm	Percentage validity
1.25	0.798±0.0130	0.658	82.45
2.5		0.581	72.80
5		0.472	59.14
10		0.299	45.44
2		0193	30.21

SUMMARY AND CONCLUSION

PLGA nanoparticles with 5-fluorouracil was prepared by double emulsification ($W_1/O/W_2$) solvent evaporation technique.3. Optimized PLGA NPs was prepared and experimental value was obtained as 217.7, the percentage of prediction error was found to be 3.51%. The prepared PLGA nanoparticle was characterized by several methods. FTIR spectroscopy revealed the chemical structure of nanoparticle. Mean zeta potential was found to be negative ie, -20 mV, SEM image showed the spherical shape and uniform size distribution. The TEM image again confirmed the size and shape of PLGA NPs. PLGA NPs showed only 3% hemolysis at 1 mg/ml concentration. Drug loading was carried out with optimized formula. Encapsulation efficiency of Drug loaded PLGA-NPs is only about 65%. Drug loading slightly increase the particle size. Drug release from PLGANPs at pH 7.4 gives initial burst release followed by sustained release pattern. The drug release kinetics exhibited 5-FU loaded PLGA NPs found to follow Higuchi model with non-flicking anomalous diffusion mechanism. Ligand biotin was conjugated to cationic polymer polyethyleneimine by using EDC and NHS chemistry and prepared biotinylated polyethyleneimine. Biotinylating was confirmed with FTIR spectroscopy and 1H NMR spectroscopy. Surface modified PLGA NPs was prepared by conjugation of biotinylated PEI(BPEI-PLGANPS). Surface modification was confirmed by FTIR spectroscopy. The Mean particle size, mean zeta potential and TE, FTIR spectroscopy confirms the surface modification. Surface modification was increased the particle size by 58.1 nm and reverse the surface charge from negative to positive (24.1). The BPEI coating was visible in TEM images. The hemolytic activity of BPEI-PLGA NPs was found to be 4.2% at 1mg/ml concentration.

Surface modification reduce the initial burst release, 5-FU showed at PH dependent release from BPEI-PLGA NPs which was showed in vitro drug release study. In vitro drug release at pH 5 was found to be more than pH 7.4. The release kinetics of BPEI-PLGA NPs was follow Higuchi model with non-Flicking anomalous diffusion. Surface modification provides a better control on drug delivery. Cytotoxicity of BPEI-PLGA NPs was studied by Brine shrimp lethality assay.LC50 value blank BPEI-PLGA NPs(blank) was found to be 831.76µg/ml and BPEI-PLGA NPs was found to be 10µg/ml. In vitro cytotoxicity studies were done by MTT assay on HeLa cell lines. IC_{50} values of the prepared nanoparticles found to be superior to free drug.

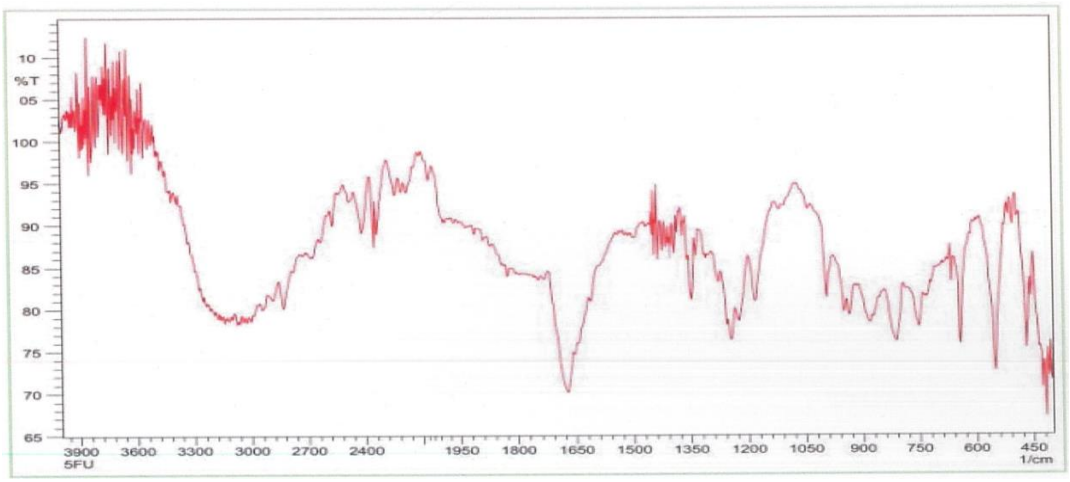
The BPEI-PLGA NPs have the greater cytotoxicity which might be attributed to the receptor mediated uptake of nanoparticles. Greater cellular uptake of BPEI-PLGA NPs might be due to the binding of the nanoparticles to biotin transport systems on the cell membrane of cancer cells. Cationic polymer coating increases the cell internalization by electrostatic interaction. MTT assay results shown that has no significant cytotoxicity. Finally, from the above data it can concluded that BPEI-PLGA NPs can act as a tumor targeted drug delivery system.

REFERENCES

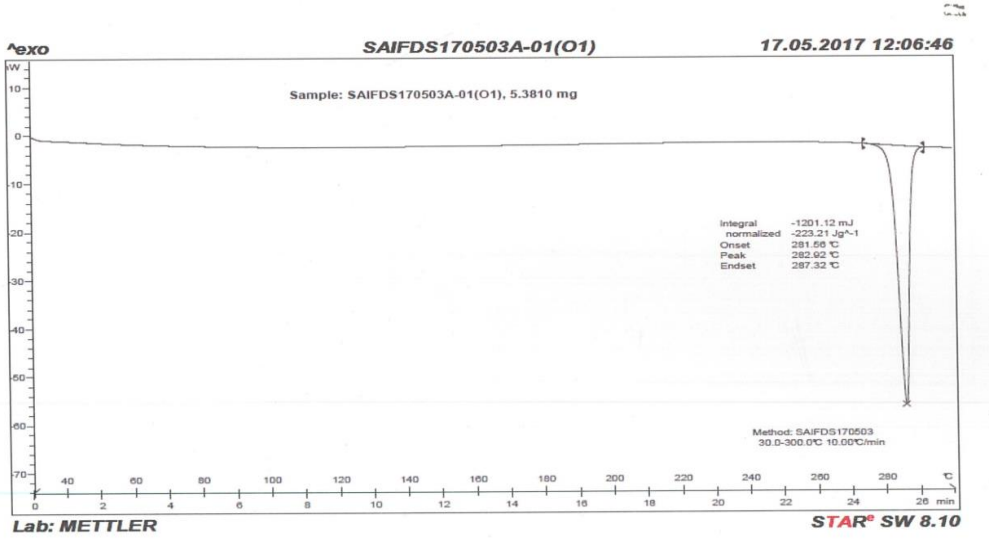
1. Zugagiotaj, Guedes.C, PonceS.FerrerI, Molina – Pinela S, Paz-AresL. (2016) Current challenges in cancer treatment, Clin ther.; 1-16.
2. Base Y.H,Park.K,(2011)targeted drug delivery to tumors: myths reality and possibility. J. control release, 153; 198-205.
3. Chen S. Zhao X, Chenj.KaznertsovaL, WongSS, (2010) Mechanism based tumor targeting drug delivery system. Validation of efficient vitamin receptor-mediated endocytosis and drug release, Bio conjugate Chem, 21: 979-987.
4. Kaki D, Jain D, Shrivastava V, Kakdi Patil A.T, (2011) Cancer therapeutics opportunities, challenges and advances in drug delivery, Journal of applied pharmaceutical sciences, 01(09): 01-10.
5. Nguyen T.K, (2011); Targeted nanoparticles for cancer therapy; promises and challenges, Nguyen j NanomedicNantechnol, 2(5).
6. Dharam S.S, Wingy, Chandana.P, Khanderej.j, Gunaseelan S, SinkoP.J, Tumor-specific targeting of an anticancer drug delivery system by LHRH peptide, PNAS, 2015; 102(36): 12962-12967.
7. Danhier F, AnsorenaE, SilvaJ.M, cocoR, BretonA.L, PreatV.A (2012) PLGA based nanoparticles: an over view of biomedical applications,J.cornet; 161: 505-522.
8. Ruoslah E.RajotteD,(2000)An address system in the vasculature of normal tissues and tumors.Annu.Rev.Immunol;18:813-827.
9. Ren WX,Hanj,UhmS,jangYj,kangC,KimJ.H;(2015); Recent development of biotin conjugation in biological imaging, sensing and targeted delivery. Chem Commun, 51(52): 10403-10418.
10. Gabizon A, HarowitzAT,GorenD,Tzemachd,MandelbaumShavitF,Qazen M M,et al.(1999); Targeting folate receptor with folate linked to extremities of poly(ethylene glycol)-grafted liposomes; in vitro studies, Biocojugate Chem., 10: 289-298.
11. Ishida O, MaryumaK, Tanahashih, IwatsuruM,SasakiH,Eriguchi M;(2000) Liposomes bearing polyethylene glycol coupled transferrin with intracellular targeting property to the solid tumors in vivo, Pharm Res., 1(18): 1042-1048.
12. FrankelA.E, PowellB.L, HallP.D, CaseL.D, Keittman R.J, (2002). Phase -1 trial of a novel diphtheria toxin/granulocyte macrophage colony-stimulating factor fusion protein (DT388GMCSF) for refractory or relapsed acute myeloid leukaemia. Clin. Oncol., 8: 1004-1013.
13. Seymour BLW, FerryDR, AndersonD, HesselwoodS, JulyanP.J, Poyner R, et al,(2002) Hepatic drug targeting:Phase-1 evaluation of polymer-bound doxorubicin, j.Clin.Oncol., 20: 1668-1676.
14. Zhang W, RanS, SambadeM, HuangX, Thorpe PE,; (2002) A monoclonal antibody that blocks VEGF binding to VEGFR 2(KDR/Flk-1) inhibit vascular expression of Flk-1 and tumor growth in an orthotopic human breast cancer model, Angiogesis, 5: 35-44.
15. Noonberg SB, BenzCC, (2000). Tyrosine kinase inhibitors targeted to the epidermal growth factor receptor sub family: role as anticancer agents, Drugs, 59(4): 753-767.
16. Smith MR, (2000). Rituximab(monoclonal anti-CD 20 antibody): mechanism of action and resistance;Oncogene, 59(4): 753-767.
17. Leonard J.P, LinkB.K, (2002) Immunotherapy of non-Hodgkin's lymphoma with HLL-2(epratuzumab, an anti-CD22 monoclonal antibody) and HuID10 (apolizumab); Semen Oncol., 29(2): 81-86.

ANNEXURES

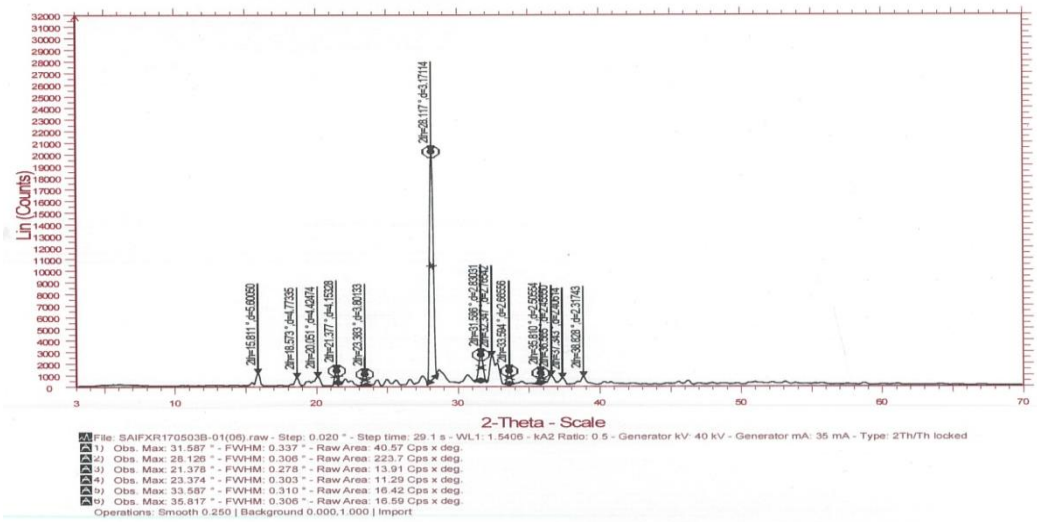
FTIR Spectroscopy of 5-Fu



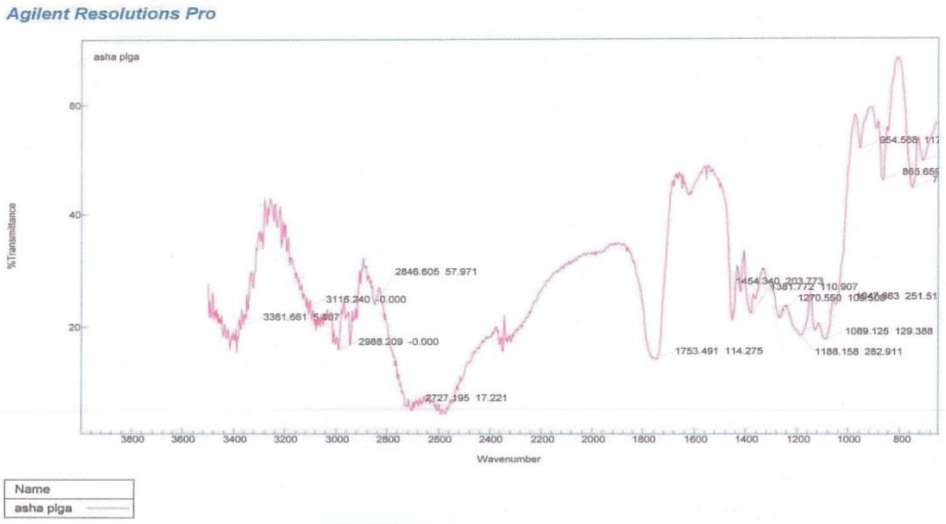
DSC data of 5- Fu



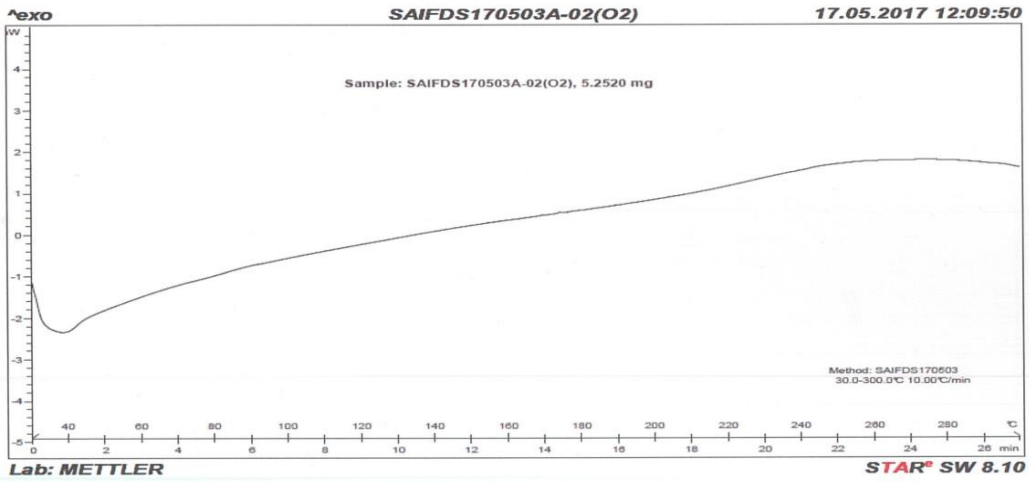
Powder of Diffraction of 5-Fu



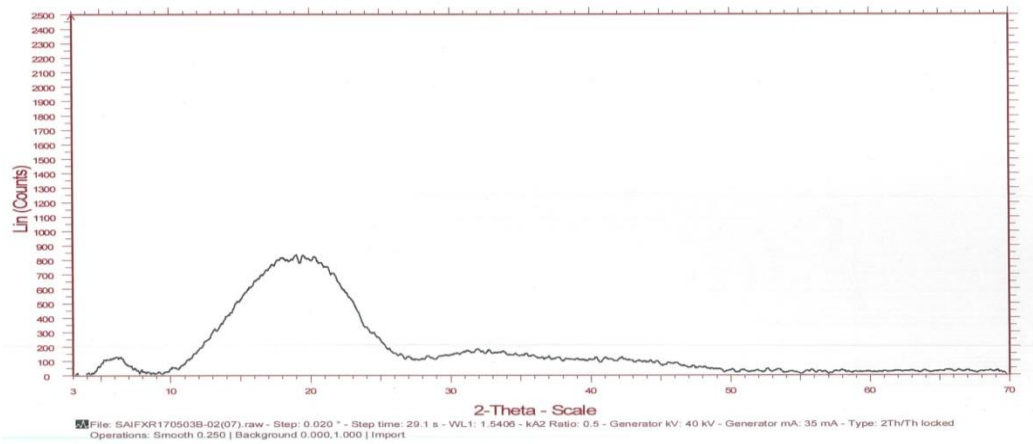
FTIR of PLGA



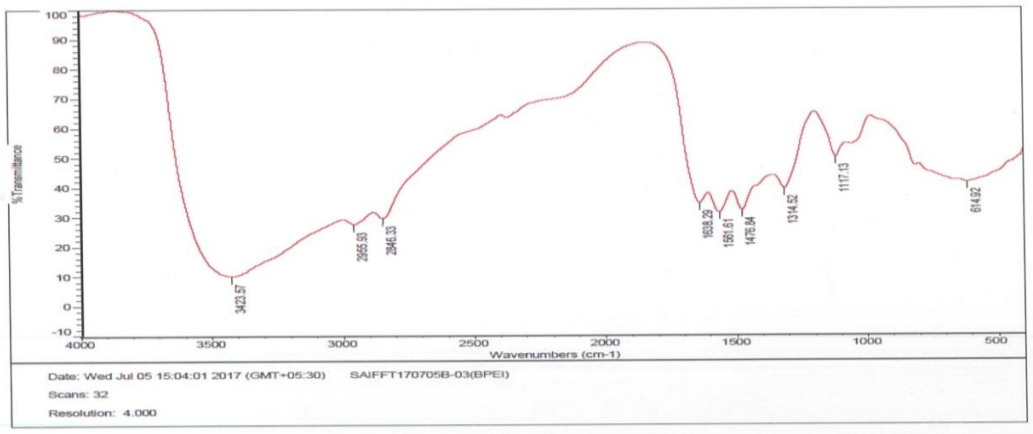
DSC of PLGA



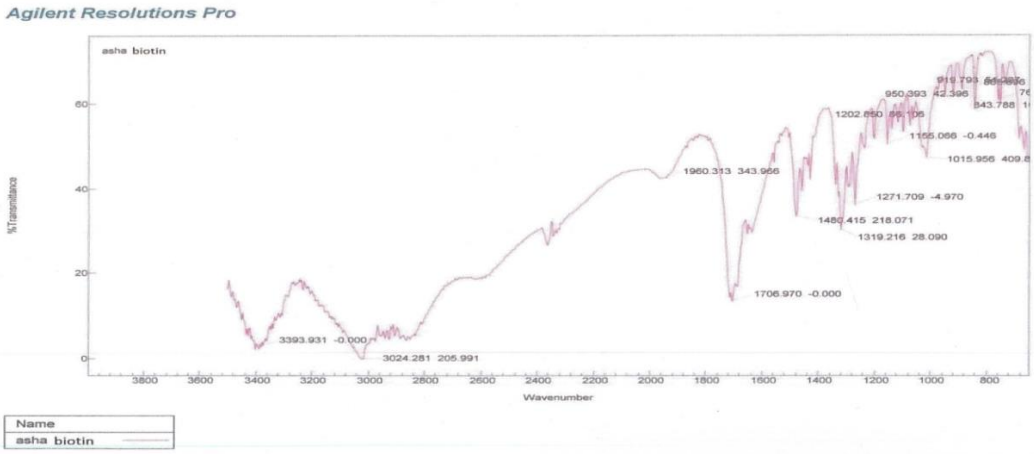
Powder X-ray Diffraction of PLGA



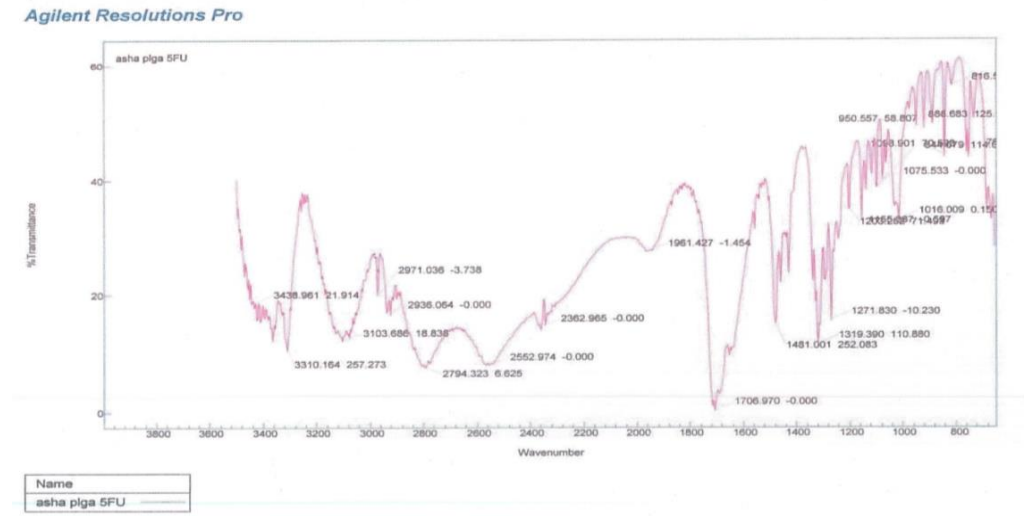
FTIR Spectroscopy of PEI



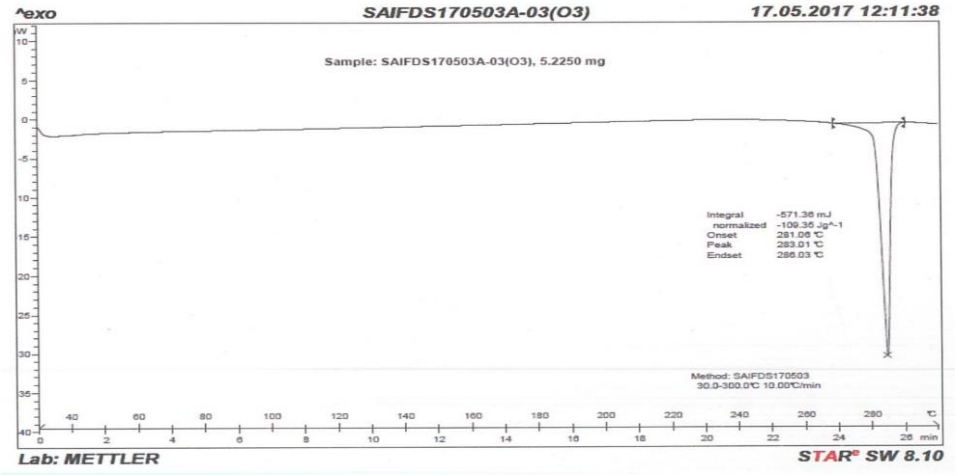
FTIR Spectroscopy of D-Biotin



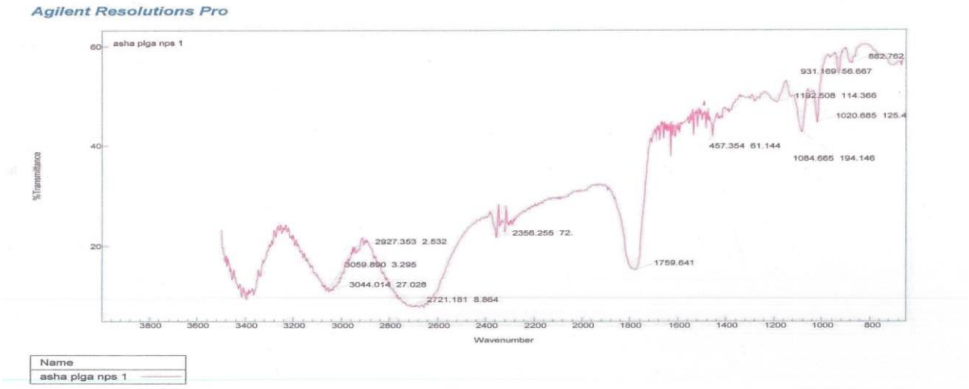
FTIR Spectro Scopy of PLGA- 5-Fu mixture



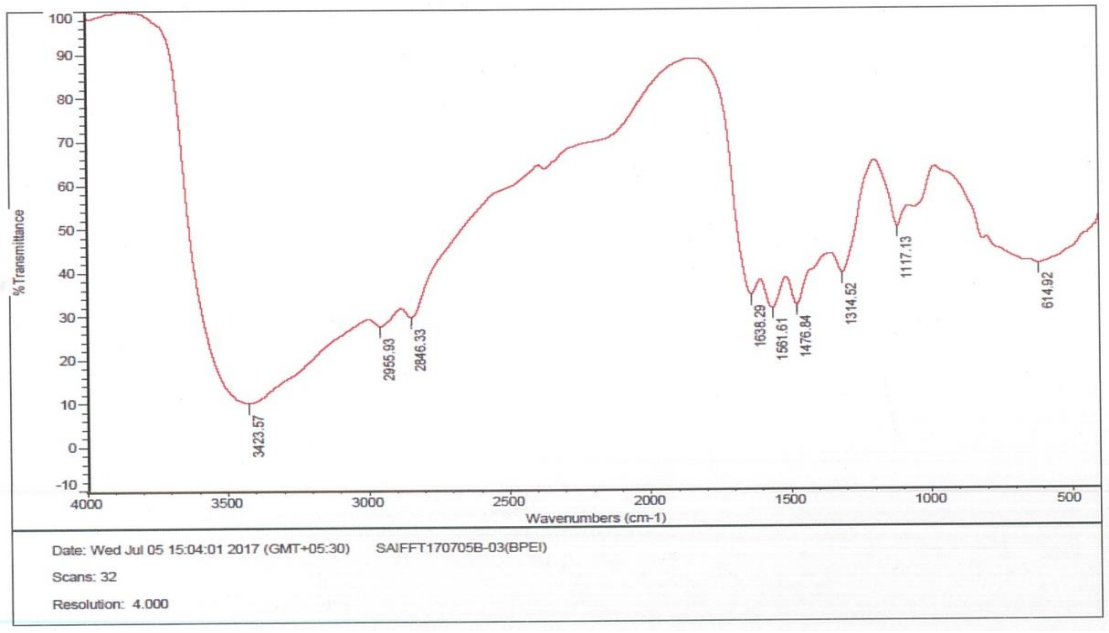
DSC of PLGA 5.Fu- Mixture



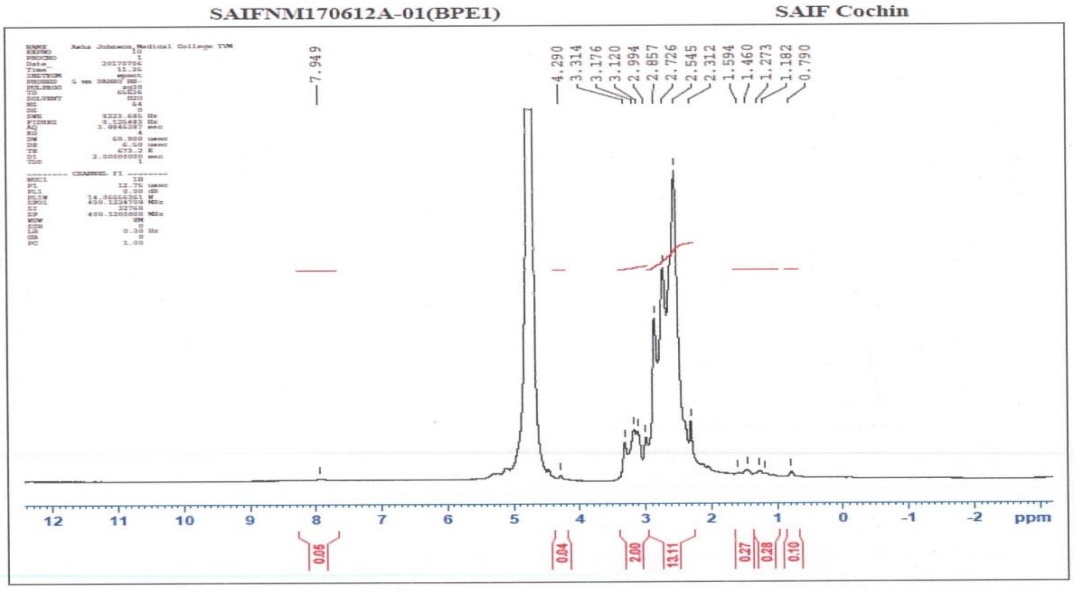
FTIR Spectroscopy of PLGA NPS



FTIR Spectro of Biotinylated Polyelthyleneimimines (BPET)



H^1 –NMR of BPEI



FTIR of Spectroscopy BPEI- NPS