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FORMULATION AND EVALUATION OF CETUXIMAB LOADED POLYMERIC NANOPARTICLES

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ABSTRACT: Nanoparticles speak to one of the appealing choices in the compelling treatment of tumor chemo-treatment. In the present work, definition, and improvement of a novel Cetuximab (MTX)- stacked biodegradable nanoparticles utilizing poly(D, L-lactide-co-glycolide) (PLGA) was done. Nanoparticles stacked with anticancer operators can effectively build sedate focus in malignancy tissues and furthermore act at the cell level, improving antitumor viability. These medication conveyance frameworks enhance bioavailability by improving fluid solvency, expanding protection time in the body and focusing on medication to a particular area in the body. The arranged nanoparticles were assessed for physicochemical properties, for example, molecule measure, zeta potential, discharge thinks about, and so forth. Molecule size of the upgraded definition was < 200 nm. Our essential outcomes exhibit that the created Cetuximab-stacked PLGA nanoparticles are discharging the medication for the delayed timeframe. The goal behind the work is to contemplate the impact of definition factors on the molecule estimate, sedate exemplification and % aggregate medication arrival of nanoparticles.

INTRODUCTION: After cardiovascular ailments, malignancy is the biggest reason for death around the world (http://www.cdc.gov.in). The word malignancy originated from a Greek word "karkinos" to depict carcinoma tumors by a doctor Hippocrates (460 - 370 B.C) [1]. Malignancy can be characterized as an uncontrolled development of typical cells in a specific piece of the body [2, 3, 4, 5]. When growth spreads to alternate parts of the body through the circulation system or lymphatic framework, this is called metastasis. A solitary malignant cell encompassed by solid tissue will reproduce at a higher rate than alternate cells.

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Once a little tumor mass has shaped, the sound tissue won't have the capacity to rival the growth cells for the insufficient supply of supplements from the circulatory system. The tumor cells will dislodge solid cells until the point that the tumor achieves dispersion constrained maximal size. (www.cancer.gov.in). The most widely recognized malignancies are anticipated to be lung and bronchus disease, bosom tumor, prostate growth, colon and rectum growth, bladder growth, melanoma of the skin, non-Hodgkin lymphoma, thyroid disease, kidney, and renal pelvis growth, endometrial disease, leukemia, and pancreatic malignancy. The quantity of new instances of disease (tumor frequency) is 454.8 for every 100,000 people for each year (in light of 2008-2012 cases) (www.nih.gov.in). The quantity of growth passings (malignancy mortality) is 171.2 for every 100,000 people for every year (because of 2008-2012 passings).

The number of individuals living past a growth finding achieved about 14.5 million out of 2014 and is required to ascend to very nearly 19 million by 2024. In 2014, an expected 15,780 kids and youths ages 0 to 19 were determined to have a tumor, and 1,960 passed on of the infection. National uses for disease mind in the United States totalled almost $125 billion out of 2010 and could reach $156 billion of every 2020 (www.nih.gov.in). Nanoparticles stacked with anticancer operators can effectively build sedate focus in malignancy tissues and furthermore act at the cell level, improving antitumor viability. The principle points of interest of nanoparticles incorporate, the amazing tumor focusing on and can escape from the vasculature through the cracked endothelial tissue that encompasses the tumor and afterward aggregate, bringing about improved porousness impacts.

These medication conveyance frameworks enhance bioavailability by improving fluid solvency, expanding protection time in the body and focusing on medication to a particular area in the body. Different medications utilized as a part of a specific sort of disease, for example, erlotinib, gefitinib, docetaxel and so on are utilized as a part of the treatment of lung tumor, while, Cetuximab, doxorubicin, paclitaxel and so on are utilized as a part of the treatment of bosom cancer. The point of the present work is to define the medication stacked polymeric nanoparticles of anticancer medication utilizing pharmaceutical trial plan. The goal behind the work is to contemplate the impact of definition factors on the molecule estimate, sedate exemplification and % aggregate medication arrival of nanoparticles [6, 7, 8, 9, 10].

MATERIAL AND METHODS:

Materials: Purac chemicals, The Netherlands liberally skilled poly (lactide-co-glycolide), PLGA (Purasorb R 85/15, Mol. wt. 10000). Poly-ε-caprolactone (PCL) (atomic weight of 40000) and Pluronic F 68 were bought from Sigma-Aldrich Chemicals, (Milwaukee, WI, USA). Cetuximab was blessing from Dabur Research Foundation (Sahibabad, U.P. India). Tc-99m was naturally eluted from molybdenum; stannous chloride dihydrate was bought from Sigma Chemicals. Triple refined water was utilized as a part of the readiness of nanoparticles.

Preparation of Nanoparticles: [11, 12] In the present investigation nanoparticles of MTX were set up by emulsification dissolvable dissipation strategy (Xua *et al*., 2005). In a word, polymer and MTX were broken up in $CH_3)_2CO$ under rapid homogenization (Polytron Mixer, Kinematica) at 1000 rpm. The polymeric arrangement was test sonicated for 2 min and gradually added to the watery stage containing surfactant arrangement utilizing fast homogenization at 7000 rpm for 10 min. Coming about O/W emulsion was again test sonicated at 40 W adequacy for 5 min in ice water shower. The emulsion was kept for mixing on an attractive stirrer at 1200 rpm for finish dissipation of natural dissolvable.

After entire vanishing of the dissolvable, the suspension was centrifuged at 20,000 rpm for 30 min. The pellet at the base was reconstituted in an answer containing mannitol as a cryo-protectant. The suspension was profound frozen at – 80 °C for 8 h and lyophilized with vacuum weight of < 50 mTorr and at a temperature of – 40 °C for 48 h. The supernatant was investigated with the expectation of complementary medication (unentrapped) utilizing as a part of the house created RP-HPLC strategy.

Characterization of Nanoparticles: The prepared nanoparticles were characterized for particle size (PS), polydispersity (PDI), zeta potential (ZP), entrapment efficiency, DSC, scanning electron microscopy (SEM), percentage yield.

a. Particle Size, Polydispersity Index and Zeta Potential: These parameters were investigated utilizing the Zetasizer Nano ZS (Malvern Instruments Ltd., UK) instrument using dynamic light disseminating (DLS) procedure. The zeta capability of a molecule is the general charge that the molecule obtains in a specific medium. In this procedure, a voltage was connected over a couple of terminals at either end of a cell containing the molecule scattering. Charged particles were pulled in to the oppositely charged anode, and their speed was estimated and communicated in unit field quality as their electrophoretic portability. The readied suspensions were weakened in MilliQ water and set in estimation cell for investigation (n = 3).

b. Determination of Drug Encapsulation Efficiency by HPLC: An analytical method of MTX has been developed for the determination of encapsulation efficiency. High-performance liquid chromatography system, HPLC LC (Shimadzu, Kyoto, Japan) equipped with UV-detector was employed for the development of the RP-HPLC method.

Encapsulation efficiency (%) = Amount of drug in nanoparticles (mg) × 100 / Initial amount of drug (mg)

Scanning Electron Microscopy (SEM): The shape and surface qualities of the nanoparticles were considered by examining electron microscopy (JSM-T20, Kyoto, Japan). A proper example of nanoparticles was mounted on a metal (aluminum) stubs, utilizing twofold sided cement carbon tape and cracked with a razor blade [4]. The examples were sputter-covered with gold/palladium for 120 s at 14 mA under argon air for auxiliary electron emissive SEM and watched for morphology at a quickening voltage of 15 kV.

Percentage Yield: The percentage yield was determined according to the formula given below Weight of nanoparticles % Yield = Weight of drug + Weight of polymer

DSC Studies: Closed vials containing drug excipient blends were charged in the stability chamber at 25 °C / 60% RH for 1 month and observed for change in physical attributes. Controlled samples were kept at refrigerated conditions (2 - 8 °C). The observations are recorded, and DSC thermograms were provided. These studies were carried out to assess the interaction between various polymers and drug and to confirm the presence of the drug in the nanoparticles either in its crystalline or amorphous form.

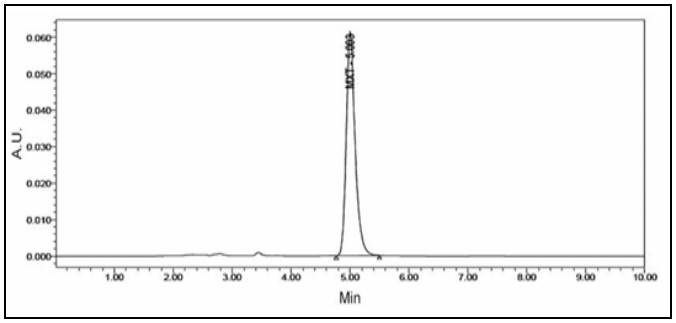

FIG. 1: REPRESENTATIVE CHROMATOGRAM OF CETUXIMAB BY HPLC

RESULTS AND DISCUSSION:

Analytical Method Development for Estimation of Cetuximab: A fast touchy diagnostic technique for the assurance of MTX has been created and approved. High performance fluid chromatography framework, HPLC LC (Shimadzu, Kyoto, Japan) furnished with UV-indicator was utilized for RP-HPLC strategy improvement **Fig. 1**. The example was investigated by the turn around stage C18 Grace Vydac (250 × 4·6 mm, 5 µ) as a stationary stage and pro to nitrile: pH 6 support arrangement (10 : 90% v/v) as a versatile stage at a stream rate of 1 ml/min. Evaluation was accomplished with bright recognition at 307 nm. The retention time of MTX was observed to be 5 ± 0.1 min. The developed strategy was approved for different parameters according to the ICH rules.

Formulation Development: From the above technique, the nanoparticles subsequently got were without dry streaming and promptly redispersible when reconstituted with Milli-Q water. The rate yield of MTX consolidated PLGA nanoparticles were gotten in the scope of 56·32 - 90·41%. Plan advancement groups were taken to get wanted molecule measure, ensnarement proficiency and % medicate discharge. Medication to polymer proportion and centralization of stabilizer were found to influence the physicochemical properties of the MTX-stacked nanoparticles. The normal molecule measure for an ideal bunch of nanoparticles was observed to be 115 - 270 nm, PDI underneath 0·50 and ZP beneath –30 mV.

The % exemplification proficiency differed in the vicinity of 8 and 16% for all the clumps. The low rate epitome effectiveness was because of contrast in osmotic weight between the two stages. The osmotic weight distinction expanded with increment in MTX stacking, prompting a crack of the lipophilic beads and a trade between the inward and external watery stages, with an ensuing loss of MTX. It is additionally conceivable that the polymer does not accelerate rapidly enough and loss of MTX happens. The dissolvable dissipation time and the idea of the consistent stage are the apparatuses to enhance encapsulation. **Table 1** and **2** indicate physiochemical properties of MTX reception. Molecule size and zeta potential dissemination of advanced MTX nanoparticles are given in **Fig. 2**.

TABLE 1: PHYSIOCHEMICAL PROPERTIES OF OPTIMIZED FORMULATION

S. no.	Drug : polymer ratio	Surfactant conc. (% w/v)	Particle size (nm)	Zeta potential (mV)	% entrapment efficiency
1	1 : 100	PVA (0·5% w/v)	199·7	–22·2	15·43

TABLE 2: FORMULATION OF CETUXIMAB BIODEGRADABLE NANOPARTICLES

Batch code	Polymers	Drug : polymer ratio	Surfactant	Surfactant conc. (% w/v)	Particle size (nm)	Zeta potential (mV)	% Entrapment efficiency
F1	PLGA	1 : 5	PVA	0·5	115·8	–23·3	9·201
F2	PLGA	1 : 10	PVA	0·5	126·9	–14·7	8·269
F3	PLGA	1 : 20	PVA	0·5	144·9	–19·7	8·810
F4	PLGA	1 : 40	PVA	0·5	164·5	–18·8	9·906
F5	PLGA	1 : 60	PVA	0·5	188·2	–26·3	9·032
F6	PLGA	1 : 80	PVA	0·5	194·6	–27·4	13·25
F7	PLGA	1 : 100	PVA	0·5	199·7	–22·2	15·43
F8	PLGA	1 : 125	PVA	0·5	233·6	–21·9	12·80
F9	PLGA	1 : 150	PVA	0·5	268·0	–24·7	14·60
F10	PLGA	1 : 100	PVA	0·25	194·6	–25·9	14·85
F11	PLGA	1 : 100	PVA	1·0	197·9	–21·1	12·44
F12	PLGA	1 : 100	PVA	1·5	211·7	–26·5	11·86
F13	PLGA	1 : 100	PVA	2·0	208·7	–23·9	10·55
F14	PLGA	1 : 100	F 68	0·5	137·9	–33·9	8·19
F15	PLGA	1 : 100	F 68	1·0	126·6	–34·7	5·08
F16	PLGA	1 : 100	F 68	1·5	127·0	–36·2	6·90
F17	PLGA	1 : 100	F 68	0·25	114·7	–40·4	8·70
F18	PLGA	1 : 100	F127	0·25	135·7	–24·6	7·88
F19	PLGA	1 : 100	F127	0·5	157·3	–30·9	8·14
F20	PLGA	1 : 100	F127	1·0	171·8	–28·4	7·69

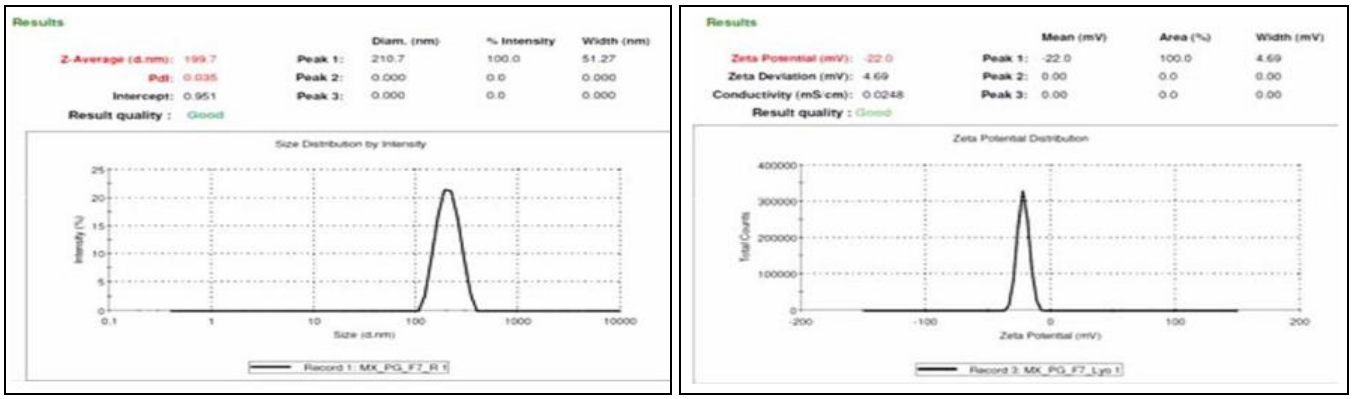

FIG. 2: PARTICLE SIZE ANALYSIS AND ZETA POTENTIAL OF OPTIMIZED CETUXIMAB NANOPARTICLES

Influence of Surfactant Concentration on Various Formulation Parameters: The molecule estimate expanded with expanding surfactant fixation even though the expansion was not huge ($p > 0·05$). Capture efficiency diminished with expanding PVA focus. In light of molecule estimate circulation and capture efficiency, the 0·5% PVA focus was chosen for additionally thinks about **Fig. 3**.

Influence of Drug:

Polymer Ratio on Various Formulation Parameters: The molecule size and capture proficiency were expanded with expanding the medication: polymer proportion. The molecule estimate expanded with the expanding grouping of PLGA broke up in a settled volume of dissolvable even though, the expansion was not huge ($p>0.05$). Slight combination of semi-framed particles may have come about because of expanded recurrence of the crash when PLGA fixation was expanded **Fig. 4**.

Surface Morphology:

Scanning Electron Microscopy (SEM): SEM revealed that the MTX nanoparticles were smooth and spherical without any aggregation **Fig. 5**.

DSC Studies: DSC studies checked the warm pinnacle of the medication and excipients connection in the details. DSC warm bend of tests was contrasted and unadulterated MTX. It was watched that DSC warm pinnacle of medication excipient mix is same as that of the unadulterated MTX. The endothermic pinnacle relating to the liquefy ing purpose of MTX somewhat moved to ±1 °C. The thermogram of the examples did not demonstrate any critical move in the endothermic pinnacle. DSC thermograms show nonattendance of trademark pinnacle of MTX in details affirming amorphization of MTX in nanoparticles **Fig. 6**.

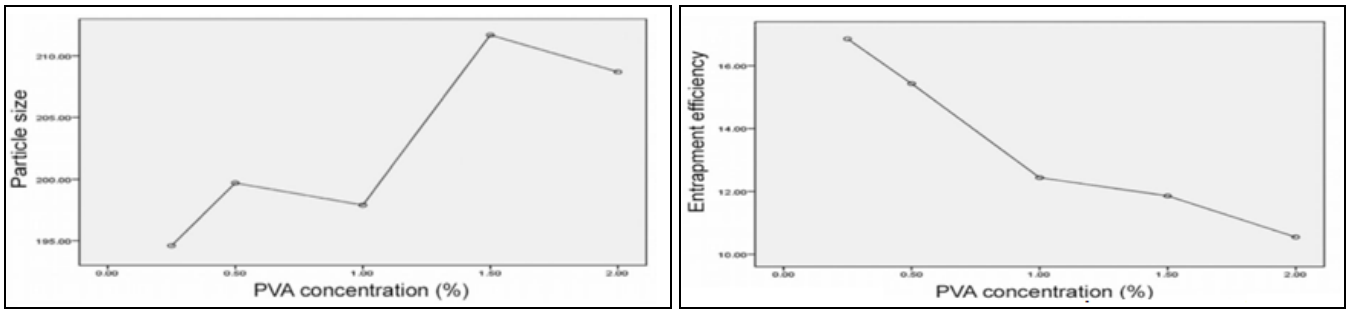

FIG. 3: INFLUENCE OF SURFACTANT CONCENTRATION ON PARTICLE SIZE AND ENTRAPMENT EFFICIENCY

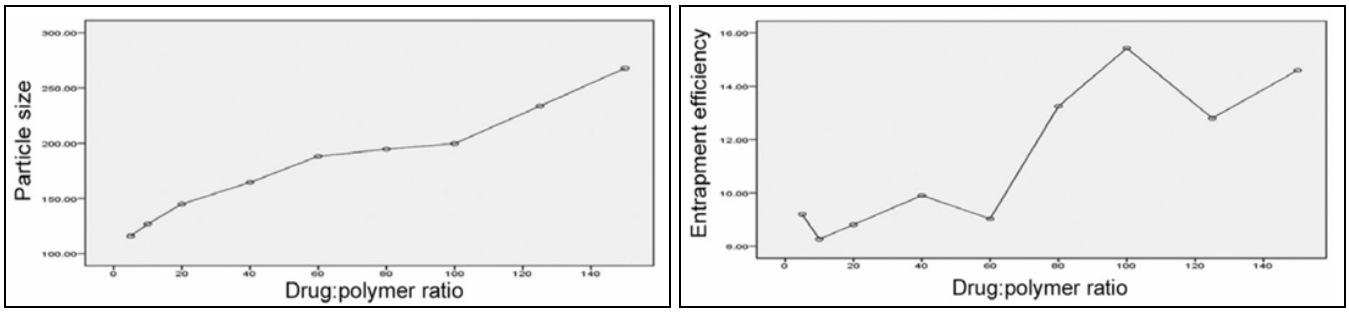

FIG. 4: INFLUENCE OF DRUG TO POLYMER RATIO ON PARTICLE SIZE AND ENTRAPMENT EFFICIENCY

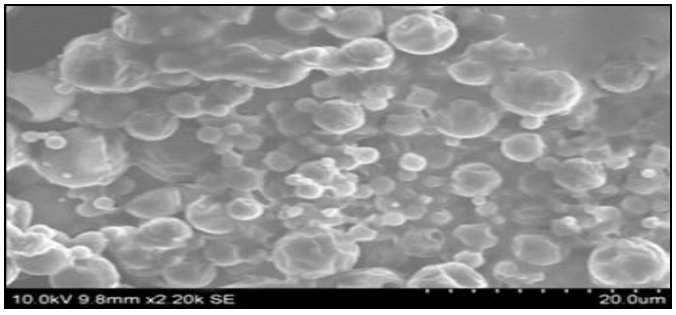

FIG. 5: SEM IMAGE OF CETUXIMAB LOADED PLGA NANOPARTICLES

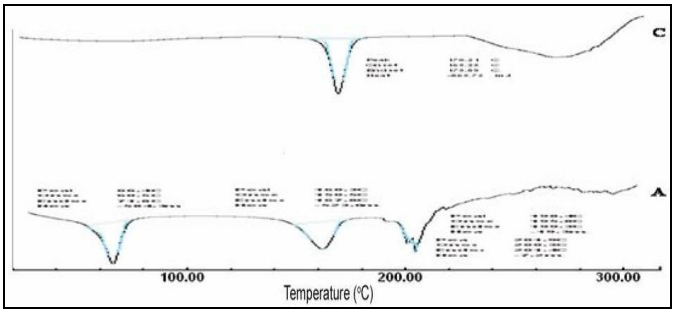

FIG. 6: DIFFERENTIAL SCANNING COLORIMETRY OF PHYSICAL MIXTURE AND FORMULATION

CONCLUSION: Our essential outcomes exhibit that the created Cetuximab - stacked PLGA nanoparticles discharging the medication for the delayed timeframe. The created plan has better anticancer movement contrasted with the unadulterated drug. From this, it is conceivable to show nanoparticles as a superior vector for the change of conveyance viability of Cetuximab.

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CONFLICT OF INTEREST: There is no Conflict of interest

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