EAS Journal of Pharmacy and Pharmacology
Abbreviated Key Title: EAS J Pharm Pharmacol
ISSN: 2663-0990 (Print) & ISSN: 2663-6719 (Online)
Published By East African Scholars Publisher, Kenya

Volume-5 | Issue-5 | Sep-Oct- 2023 | DOI: 10.36349/easjpp.2023.v05i05.001

OPEN ACCESS

Original Research Article

Formulation and *In vitro* Cytotoxicity Studies of Avastin Nanoparticle Mediated Targeted Nose to Brain Drug Delivery System

Mrugendra B. Potdar[1*], Neetesh K. Jain[2]

[1]Research Scholar, Department of Pharmaceutics, Oriental University Indore - 453555, Madhya Pradesh, India
[2]Department of Pharmacology, Oriental University, Indore - 453555, Madhya Pradesh, India

Article History
Received: 29.07.2023
Accepted: 05.09.2023
Published: 11.09.2023

Journal homepage:
https://www.easpublisher.com

Quick Response Code

Abstract: Nanoparticles (NPs) occupy a superior place among the recent approaches experimented to deliver drugs into brain by circumventing the BBB such as chemical modifications. The Emulsification PEG-PLGA nanoparticles carrying drug were made using the solvent evaporation method. In summary, PEG-PLGA was dissolved in acetone (50 mL) and PLGA was dissolved in PEG-PLGA (50:50, 7-11 mg/mL). The Chitosan-Avastin conjugates were created in the lab in accordance with the procedures and guidelines outlined in Chitosan (3.25 m mol) was hydrated in 1 N HCl, then the appropriate amount of distilled water was added. After adding NaOH (5N) to the reaction mixture to get the pH down to 5, Avastin (6.48 mmol) was added while the mixture was continuously stirred. In-vitro studies the basic aim is to check the effect of various drugs on various cancer cell lines. For testing purpose a large number of human cancer cell lines have been screened. Cells are cultivated in 96-well culture plates, and the rate of cell multiplication, which determines the rate of cell growth, is indirectly measured by the dye's color intensity, which is directly inversely proportional to the number of cells present. Using doses between 10 and 100 g/mL, the MTT test was performed to examine the cytotoxicity various polymer ratios and formulations on human lung cancer lines-A549. For the determination of the percentage of viability of various samples, the cancer cells without receiving therapy were utilized as the control.

Keywords: Nose-to-brain delivery, Emulsification Solvent Evaporation Method, Nanoparticles, Avastin, MTT assay, *In vitro* cytotoxicity.

1. INTRODUCTION

Nanoparticles (NPs) occupy a superior place among the recent approaches experimented to deliver drugs into brain by circumventing the BBB such as chemical modifications and prodrug approaches, drug targeting with the aid of magnetic materials, or drug carrier systems such as liposomes. Solid colloidal particles with a diameter between 1 and 1000 nm are known as nanoparticles. The BBB can be crossed and medications can be transported to the precise locations in the brain where they are required by coating or encapsulating them in nanoparticles. The peripheral toxicity of drugs can be decreased by using nanoparticles that release drugs slowly into the circulation [1].

Nanoparticulate drug delivery systems are accepted as an attractive method due to the properties of nanoparticles such as small size and biodegradability and biocompatibility of the materials used in its preparation. The small size of nanoparticles permits them to infiltrate through the sites of inflammation or penetrate into smaller capillaries, and is generally taken up efficiently by the cancer cells which provide therapeutic concentrations of drugs at the target sites. Targeted delivery using nanoformulations can be achieved by either active or passive approaches. Nanoparticles can be actively targeted by conjugating them to a cell-specific ligand small size and engineered surface properties of the nanoparticles guide them to the target sites passively. This property is responsible for the enabling the nanoparticles to deliver drugs across several biological barriers to the target site [2].

2. MATERIALS AND METHODS

2.1 Materials:

All the chemicals used in research work were procured from Chemdyes Corporation Rajkot (Gujarat).

***Corresponding Author:** Mrugendra B. Potdar
Research Scholar, Department of Pharmaceutics, Oriental University Indore - 453555, Madhya Pradesh, India

2.2 Methods:

Solubility Studies:

Supersaturated solutions are solutions with an excess of solute in the solvent. In 0.1N HCl, the medicines' phase solubility was evaluated. Avastin's solubility was tested by dissolving an excess of 10 mg of the drug in 10 ml of solvent in vials. The samples were maintained in equilibrium for 24 hours in an incubator at 37°–0.50°C with periodic shaking. After being filtered with Whatman filter paper, the supernatant from the vials was ready to be examined with a Shimadzu UV-Visible spectrophotometer at the appropriate wavelength. Although the shake-flask approach is time-consuming, it is the most accurate way to test solubility [3].

Drug Excipient Compatibility Studies:

FTIR conducted research to determine whether a medicine and its excipients were compatible. The FTIR spectrum of a pure drug obtained with excipients was compared to the FTIR spectrum of a pure drug obtained without excipients [4].

Synthesis of Drug-PEG–PLGA Conjugates by Emulsification Solvent Evaporation Method:

The synthesis of PEG-PLGA nanoparticles containing medicines was done using the emulsification solvent evaporation method. In summary, PEG-PLGA was dissolved in acetone (50 mL) and PLGA was dissolved in PEG-PLGA (50:50, 7-11 mg/mL). A 50 mL aqueous phase and 1% Polyvinyl alcohol (PVA) surfactant were combined with this organic phase to create an emulsion. To create an O/W emulsion, the mixture was probe-sonicated for 60 seconds at 5 amplitudes using a Cole-Phrmer 750-watt Ultrasonic Homogenizer with Temperature Controller. Using a magnetic stirrer, the O/W emulsion was agitated for 4 hours at room temperature until the organic phase had completely evaporated, producing nanoparticles. Ultracentrifugation was used to separate the nanoparticles for 15 min. at 50,000 g. After being re-suspended, the PLGA nanoparticles were rinsed with water and collected [5].

Table 1: Formulation table of Nanoparticle

Sr No	Material	F-1	F-2	F-3
1	Polymer Concentration (PEG-PLGA) (mg/ml)	5	10	15
2	Drug Concentration (AVASTIN) (mg/ml)	0.5	1	2
3	Ratio of solvent to Water	0.1	0.25	0.45

Synthesis and Characterization of Chitosan-Catechol Conjugates:

Avastin conjugated chitosan is a prime option for use in polymeric drug delivery systems that target biological mucosal lining due to its strong mucoadhesive characteristics. Chemical conjugation techniques are used to create the conjugates of chitosan, carmustine, and avastin [6].

Synthesis of Drug-Chitosan Conjugation:

The Chitosan-Avastin conjugates were developed in the lab in accordance with the procedures and guidelines outlined in Chitosan (3.25 m mol) had been hydrated in 1 N HCl, then the appropriate amount of distilled water was added. After adding NaOH (5N) to the reaction mixture to get the pH down to 5, Avastin (6.48 mmol) was added while the mixture was continuously stirred. EDC (6.49 mmol) was thoroughly dissolved in a 1:1 v/v solution of ethanol before being gradually mixed in with drops of distilled water. The reaction mixture was then dialyzed in distilled water that had been acidified with HCl (pH 5) for two days. The finished product was desiccated and freeze dried [7].

***IN VITRO* CYTOTOXICITY STUDIES**

Chemicals Required

The Chemicals used in the present study along with their sources are outline below: Tris buffer (Sigma), Dimethyl sulfoxide (DMSO) (Sigma), Ethylenediamine tetraacetic acid, disodium salt (EDTA) (HiMedia), Fetal Bovine Serum (FBS) (Sigma), Glacial acetic acid (HiMedia), Isopropyl alcohol (sisco), Penicillin (Sigma), Streptomycin (sigma), Phosphate Buffer Saline (PBS) (Sigma).

Reagents:

Complete Growth Medium: 10% Penicillin (100 units/ml, before use) was added to the growth medium to make it a complete growth medium.

PBS, or phosphate buffer saline: Melt 9.6 grams of it. with addition to washing the cells with distilled water in an autoclave, PBS was used to make solutions of Trypsin EDTA and Penicillin.

Trypsin EDTA: Under sterile conditions, 50 mg of trypsin (0.05%) and 20 mg of dissolved EDTA salt (0.02%) were dissolved in PBS. While sub-culturing and dividing the cell line, trypsin EDTA is employed to separate the cells.

Penicillin Solution: 625 g of penicillin, or 100 units, per milliliter of PBS. To prevent contamination, penicillin was added to the RMPI medium.

Gentamycin Solution: 50 g/ml of the drug was dissolved in PBS. The medium used in the experiment included Gentamycin.

Human Cancer cell Lines: - Cancer can be of varying types; in fact cancers are classified by the way they

infect the particular organ of the body. Almost every part of human body is cancer prone. In-vitro studies the basic aim is to check the effect of various drugs on various cancer cell lines. For testing purpose a large number of human cancer cell lines have been screened. The different cancer cell lines available at IIIM, Jammu for testing herbal drugs are as follows;

Table 2: Represents list of cell lines related tissues, medium, require inoculation density

Tissue	Cell lines	Medium	No. of cells/well	Doubling Time	Positive control
Leukemia	HL-60	RPMI-1640	10,000	28.6	Camptothecin
	MOLT-4	RPMI-1640	30,000	27.9	Camptothecin
	THP-1	RPMI-1640	10,000	35.5	Camptothecin
Lung	A-549	DMEM	7,500	22.9	Doxorubicin
	HOP-62	RPMI-1640	20,000	35.5	
Colon	COLO-205	RPMI	12,000-15,000	23.8	5-Fluorouracil, BEZ-235,
	HCT-116	DMEM	5,000	17.4	5-Fluorouracil, BEZ-235,
Melanoma	M-14	MEM	10,000	26.3	
Breast	MCF-7	MEM	10,000	25.4	BEZ-235
	MDA-MB-231	RPMI-1640	15,000-20,000	41.9	BEZ-235
Pancreas	MIA-PaCa	DMEM	2000-5000	40	BEZ-235
	PANC-1	DMEM	3000	28	
	AsPC-1	RPMI-1640	5000	31	

Cell line sources:

The National Cancer Institute (NCI), Frederick (USA), provided the original supply of human cancer cell lines in frozen Tate (dry ice) in cryovials, and certain cell lines were acquired from the National Centre for Cell Science (NCCS), Pune (India), in culture flasks.

Cell Culture and Maintenance for Adherent Cell Lines:

Human cancer cells were cultured in tissue culture flasks with full growth media at 37°C in an environment of 5% CO2 and 90% relative humidity in a carbon dioxide incubator. Daily checks were made to ensure optimal cell development. When the color turned yellow, the cells' medium was altered. The medium in the flask was aspirated using a pipette and discarded to change the medium. Under sterile conditions, fresh medium was added to the culture flask. Flasks were correctly labeled and placed in a CO2 incubator. At the sub-confluent stage, cells were sub-cultured based on the mass doubling time of the cells [8].

Suspension cell culture and maintenance Cell lines:

In tissue culture flasks with complete growth media at 370C in an environment of 5% CO2 and 90% relative humidity, human cancer cells were cultivated. Daily checks were made to ensure optimal cell development. When the color turned yellow, the cells' medium was altered. The media in the flask was aspirated using a pipette and kept in a centrifuge tube to change the medium. Next, centrifuge for 8 to 10 minutes at 1200 rpm. The cell pellet was reconstituted with brand-new complete growth media after the supernatant was discarded. The cell suspension was then transferred to a brand-new culture flask filled with a brand-new media and kept under sterile conditions. The flask was appropriately labeled and kept incubating in CO_2 incubator. Depending on the was doubling time of the cells, sub-culturing of cells was done.

Subculture of the Cell Lines:

It entails removing the cells from the culture flask's growth surface and reinoculating them in fresh medium in a new culture flask, such as a TCF-25, TCF-75, or TCF-175 flask (depending on the quantity of cells). One day in advance, the medium in the flask used for sub-confluent growth was changed. The entire flask's medium was removed and thrown away. PBS was used to wash the cells. Just enough Trypsin-EDTA (pre-warmed at 37°C) was applied to form a thin layer, and it was then incubated for 5 minutes at that temperature. Cells were observed under microscope. Then add nearly 2 ml of media in the flask and pass through pipette to make single cell suspension. The cells were then transferred in the centrifuge tube and were centrifuged at 1000 rpm for 5 minute. After centrifugation discard the medium and add fresh medium about 2 ml in the centrifuge tube asepticall [9]. Resuspend the cells in the medium by using pipette. Add medium in the new flask followed by the addition of cell suspension (about 100- 300ul). This is referred to as cell line splitting. The flasks are kept in an incubator with a 37°C and 5% CO2 atmosphere. It is important to take care not to use more Trypsin-EDTA or leave the cells in the solution for prolonged periods of time as this could harm the cells. To reduce damage, add sufficient media to the trypsinized cells as soon as possible [10].

Freezing and Thawing:

Long-term cell cultures may experience genotypic or microbial contamination, which could result in the loss of defined cell lines. Cells can be

frozen and stored (cryo-preserved) practically indefinitely at a very low temperature in liquid nitrogen to prevent cell loss. (-196^{0}C).

Cryopreservation:

Consider the jars containing the cells in the log stage of development. No one is surprised when the cells are trypsinized. By passing the cells through a pipette, prepare the single cell solution. Pour the cell suspension into the cleaned axis cylinders and rotator, then spin the machine at 1000 rpm for five minutes at 0 °C. Dispose of the supernatant. Pipette the pellet into a single cell solution after adding the freezing medium. Fill the cryovial suspension with the single cell suspension to 2/3 of its capacity. Cryovials are stored in cryocontainers, which contain isopropanol to prevent unexpected temperature drops that could injure the cells. Keep the cryocontainer in a deep cooler at -80°C [11]. The cells can be kept in the cryoprotectant at liquid nitrogen (-196°C) for virtually indefinitely. In order to prevent the isopropanol from spilling out and damaging the cells, the container should be set down on a flat surface. When inserting the cryovials, the cryocontainer in which the cells are to be housed must be at normal temperature. By doing this, a sudden drop in temperature won't harm the cells by causing ice crystals to develop while the cells are still in the subconfluent stage [12].

Sterility Test of the Media:

This can be done to check any bacterial or fungal contamination in the media. About3ml of freshly prepared media was incubated at 37°C for 24-48hrs.After incubation, media is observed under inverted microscope for any bacterial or fungal growth. If there is any contamination, media is discarded as it cannot be used for culturing the cells.

Counting of the Cells:

The hemocytometer, sometimes known as a hemocytometer, is a tool that was initially made for counting blood cells. Today, it is also used to count different kinds of minute particles and other types of cells. Louis-Charles Malassez [8] is credited with creating a thick glass microscope slide with a rectangular depression that creates a chamber makes up the hemocytometer. This chamber has been laser-etched with a grid of parallel lines. The intricately constructed mechanism allows for the precise measurement of both the chamber's depth and the area bounded by the lines. Therefore, it is possible to count the number of cells or particles in a specific volume of fluid in order to determine the concentration of cells in the fluid overall.

Method of Calculating the Cells

Capillary activity entirely fills the chamber with the Sample after a small volume of media containing cells is placed on the haemocytometer and covered with cover glass. The number of cells in the chamber can be counted while looking at it under a microscope. As long as cells can be identified visually, they can be counted independently [13]. The density or concentration of the cells in the mixture from which the sample was obtained is determined by the number of cells in the chamber.

Preparation of Test Material

Stock Solution:

One day before they are needed, stock solutions are made. Create a 20 mg/ml stock solution. Dimethyl sulphoxide (DMSO) is frequently employed as a solvent to dissolve test materials, however because it is harmful at high concentrations, we limit its usage to no more than 0.5%.

Determination of *In-Vitro* Cytotoxicity:

Mammalian cell lines are cultured in environments where they are actively developing and going through mitosis for cytotoxicity testing. Cells are cultivated in 96-well culture plates, and the rate of cell multiplication, which determines the rate of cell growth, is indirectly measured by the dye's color intensity, which is directly inversely proportional to the number of cells present. The cytotoxicity assay compares a cancer cell line's rate of proliferation in the presence and absence of the test drug, often after a set amount of time. Ideally, several different cancer cell lines are used.

Screening of compounds with MTT Assay: -

Cytotoxicity assay based on MTT was developed by Alley *et al.,* It is colorimetric assays,

Making Cell Suspension:

To produce an adequate number of cells, the selected human cancer cell line is cultivated in tissue culture flasks at 370°C, in an atmosphere of 5% CO2 and 90% relative humidity. Choose a flask with a growth stage that is sub-confluent. After trypsin-EDTA treatment, extract the cells. Use a hemocytometer to count the number of cells per milliliter of suspension, In the cell suspension, adjust the cell count to 10,000 cells per 100 l [14]. Using a multichannel, add 100 l of cell suspension to each well of a 96-well plate. For 24 hours, incubate the plates at 37 °C in an environment with 5% CO2 and 90% relative humidity.

Addition of Test Material:

Finish of 48 hours before 4 hours 20µl of MTT color (2.5mg/ml) was added per well I. e. each well gets 5ug of MTT color. MBrood the cells with color for 3-4 hours. After 4hrs.

Figure 1: 96 well plates used for MTT Assay reading

Presently supernatant is disposed of and 150 Of DMSO is included each well. Plates were delicately shaken on a plate shaker for 5 mints. If different concentrations of the same test sample were used, they should be in the successive wells in the same plate. After 24 hours the 100 µl of working solution of each test material is added to the wells of 96 well plates (for adherent cell line) and test material added within 4 hours for suspension cell line.

Calculation of Cell Viability:

The 50% inhibitory concentration (IC50), which can be stated as a molar concentration or on a mass per volume basis such as micrograms per milliliter or milligrams per liter, is how drugs' anti-proliferative effect is quantified. The former manner of expression is preferred, and it is simple to calculate using the molecular mass of a 50% reduction in the baseline or control level of proliferation. The OD value of the appropriate control wells in the MTT test plate serves as the starting point for calculating the IC50 value because the MTT assay's readout is OD [15]. The correct control OD value is necessary for this analysis and should be precisely matched for the dilution of the solvent used to make the stock solution of the test agent [16]. By dividing the observed OD value by the appropriate control and multiplying by 100, as indicated in the following equation, one can determine the percent inhibition at each concentration of the test agent using the control OD values: = (AT-AB) / (AC-AB) X 100 = % viability Where AT = Absorption of drug-treated cells AB stands for absorbance of blank (cell-free) Absorbance of untreated (control) As a result, cell survival is equal to 100 percent when cell growth is inhibited by cytotoxicity.

Determination of IC_{50} Value:

The FDA defines IC50 as the amount of a medication necessary to produce 50% inhibition in vitro. In our study, the IC50- is the medication concentration at which 50% of the population of cells dies. In order to determine whether a chemical is hazardous to cell lines during initial screening, we adopt a cutoff of 50% cell growth inhibition [17]. The dose response curve between the log of the drug concentration and the percentage growth inhibition was plotted to calculate the IC50. The graph was drawn with the logarithmic drug concentration on the X-axis and the percentage of cytotoxicity or suppression of cell growth on the Y-axis. The drug concentration at the 50% location on the Y axis was calculated as IC50 [18].

The relationship should be sigmoidal, log drug concentration on the x-axis and 'response/measurement' of the Y-axis [19].

3. RESULT AND DISCUSSION

3.1 Identification of Drug

By Infrared Spectroscopy:

The finely ground sample in KBr dispersion packed into a disc should only show maxima at wavelengths that correspond to a similarly prepared working standard in terms of its infrared absorption spectra.

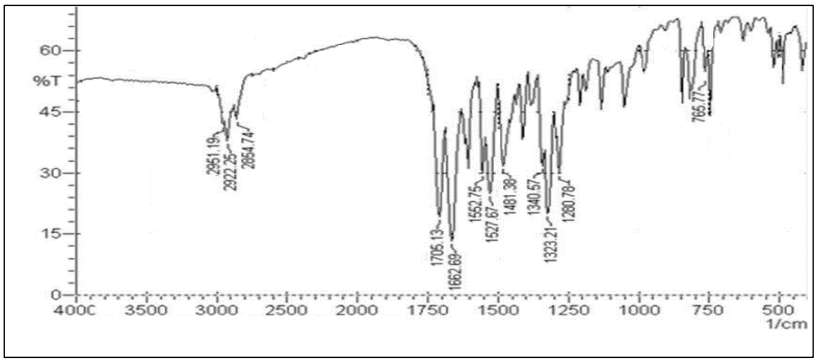

Figure 2: FTIR spectrum of Avastin

Table 3: Wave number of functional groups of Avastin

Sr. No.	Wave number	Carmustine Functional group
1	1100-1200 cm^{-1}	C-N
2	1200-1300 cm^{-1}	PO_2
3	1300-1400 cm^{-1}	C-N
4	1400-1450 cm^{-1}	C-C
5	1500-1600 cm^{-1}	Amide-II
6	1600-1650 cm^{-1}	Amide-I
7	1745-1725 cm^{-1}	C=O (ester)

Determination of Analytical Wave length:

The absorbance maxima were noticed at 280.0 nm when the standard carmustine solution (50 g/mL) was scanned in a 1.0 cm cell against a solvent blank and spectra were recorded.

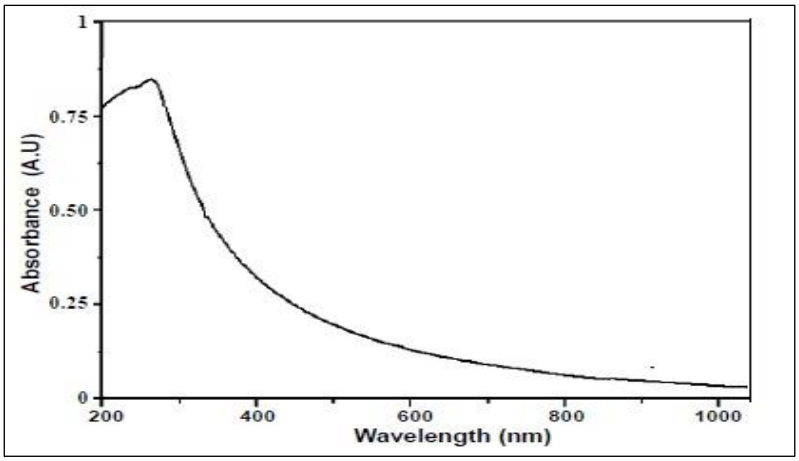

Figure 3: UV spectrum of avastin in 6.8 phosphate buffer

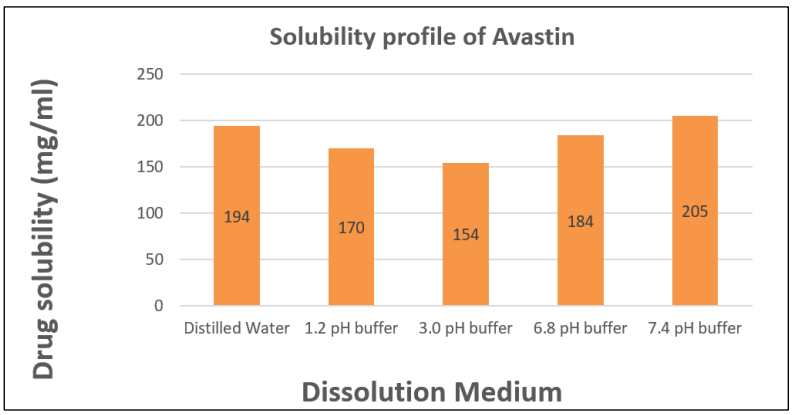

Figure 4: Solubility profile of Avastin

***In Vitro* Drug Release Study:**

In vitro drug release of carmustine from PLGA NPs was studied by dialysis bag diffusion technique. Cumulative drug release from pegylated NPs formulation (F1 to F3) had shown maximum release about 96.11±0.38 in the phosphate buffer pH 6.8, respectively after 24 h. The cumulative percentage drug release of batch F1 to F3 is represented in Table 2. The surface modification of optimum formulations was achieved with PEG 6000 duets its diverse characteristics such as hydrophilic nature, non-toxicity, unavailability of antigenic and immunogenic properties. The PEGylation of NPs (F1) exhibited slow release in comparison to the unmodified NPs.

Table 4: *In Vitro* Drug Release Study of Nanoparticles

Time Interval (h)	F1	F2	F3
	% Cumulative Drug Released ± Standard Deviation		
0.5	11.01±0.44	16.13±0.37	17.26±0.57
1	13.77±0.31	19.76±0.26	21.84±0.73
2	19.89± 0.5	27.23±0.5	28.39±0.61
4	25.64± 0.3	31.46±0.33	33.57±0.29
6	30.73± 0.6	37.23±0.45	38.89±0.43
8	35.94±0.59	41.02±0.62	43.84±0.3
12	59.14± 0.81	59.11±0.32	61.83±0.47
18	88.17 ± 0.38	87.24±0.43	90.53±0.26
24	93.25± 0.29	95.96±0.41	96.11±0.21

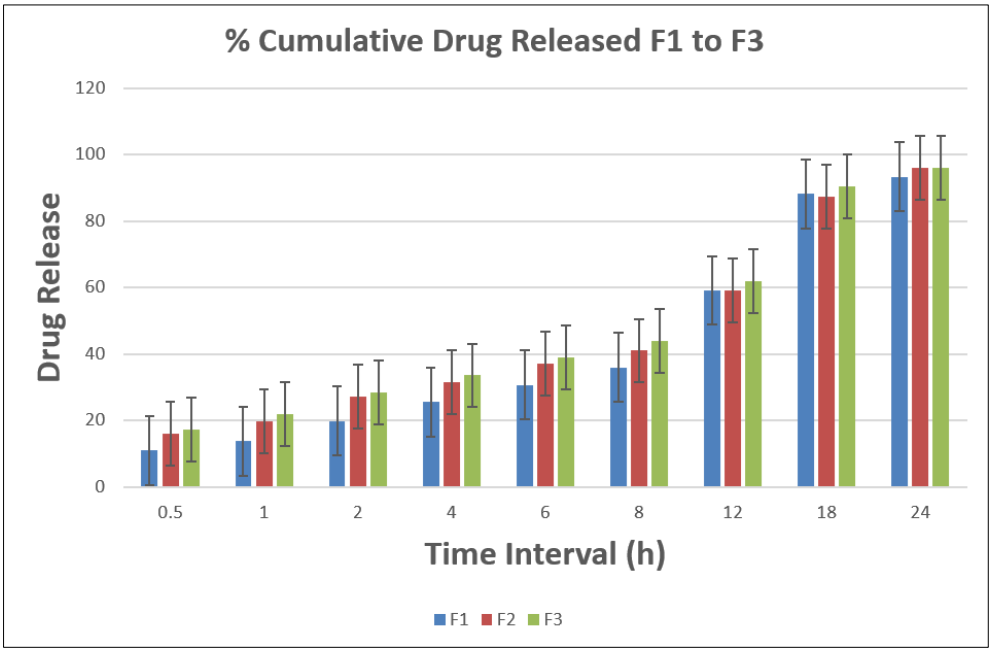

Figure 5: Cumulative percentage drug release of batch F1 to F3

***In Vitro* Determination of In-Vitro Cytotoxicity:**

In vitro Cytotoxicity of formulation F1 to F3 central composite designed Polyer loaded nanoparticles (F1 to F3) and surface modified NPs (F1 to F3) prepared by nanoprecipitation technique were studied. MTT assay was used to investigate the cytotoxicity of Polymer ratio and formulations on Human lung cancer lines-A549 by using concentrations range of 10-100 µg/mL. The cancer cells without treatment were used as control for estimation of percentage viability of different samples. Among all the formulations, polymer loaded all formulation showed the Cytotoxicity.

Table 5: IC_{50} Values of the Nanoparticles (F4 to F6)

S. NO.	SAMPLE COAD	CONC. (µM)	Tissu cell Line							
			MCF-7		HCT-116		A-549		MDAMB-231	
			% INHIBITION	IC_{50}	% INHIBITION	IC_{50}	% INHIBITION	IC_{50}	% INHIBITION	IC_{50}
1	F-1	10	85.36	6.91 µM	87.54	4.12 µM	83.17	5.00 µM	82.91	5.16 µM
		50	91.51		94.19		90.31		90.19	
		80	92.86		95.86		91.59		94.66	
		100	95.95		96.27		92.80		96.57	
2	F-2	10	88.21	5.78 µM	81.50	8.34 µM	82.54	4.47 µM	90.47	5.60 µM
		50	91.58		91.08		89.67		94.74	
		80	95.21		96.91		91.35		96.86	
		100	97.37		97.06		92.33		97.43	
3	F-3	10	92.96	5.56 µM	86.48	8.41 µM	83.28	3.36 µM	92.28	1.70 µM
		50	91.17		89.07		88.90		94.12	
		80	95.08		95.36		96.38		95.31	
		100	97.97		96.60		96.98		96.73	

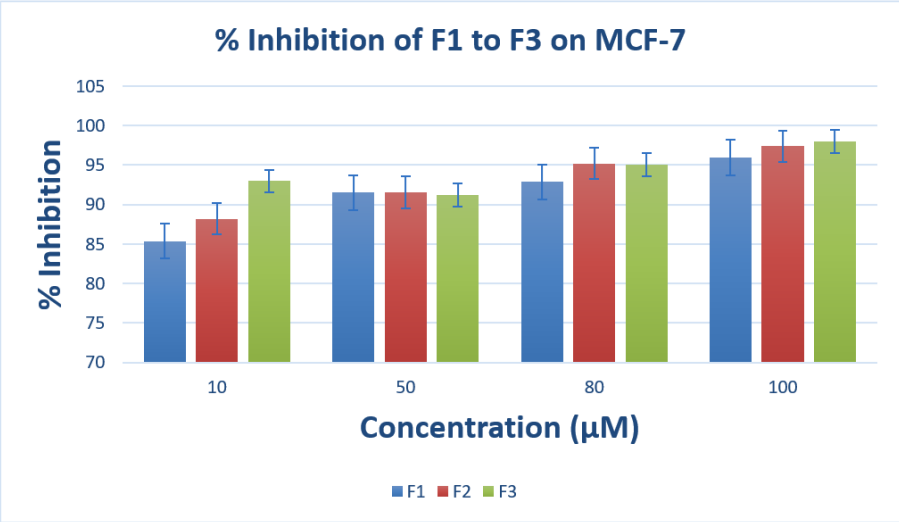

Figure 6: %inhibition of (F1-F3) on MCF-7

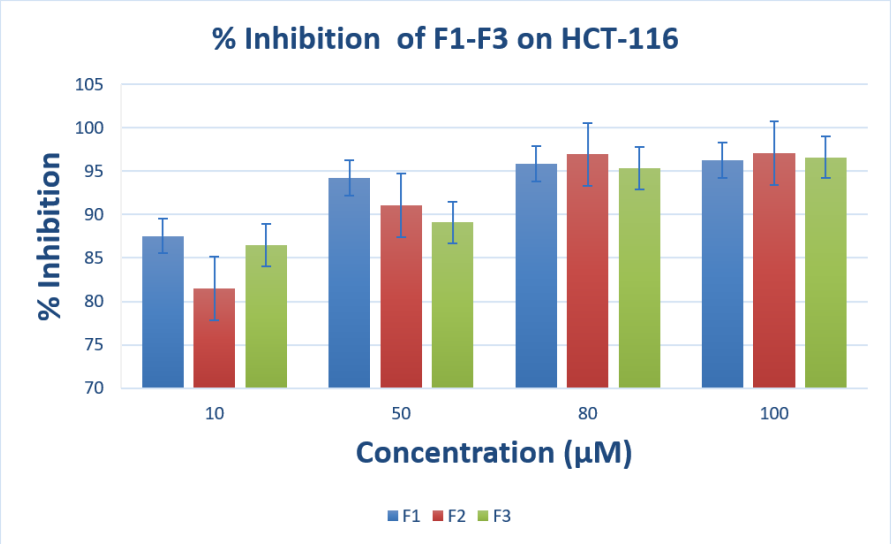

Figure 7: %inhibition of (F1-F3) on HCT-116

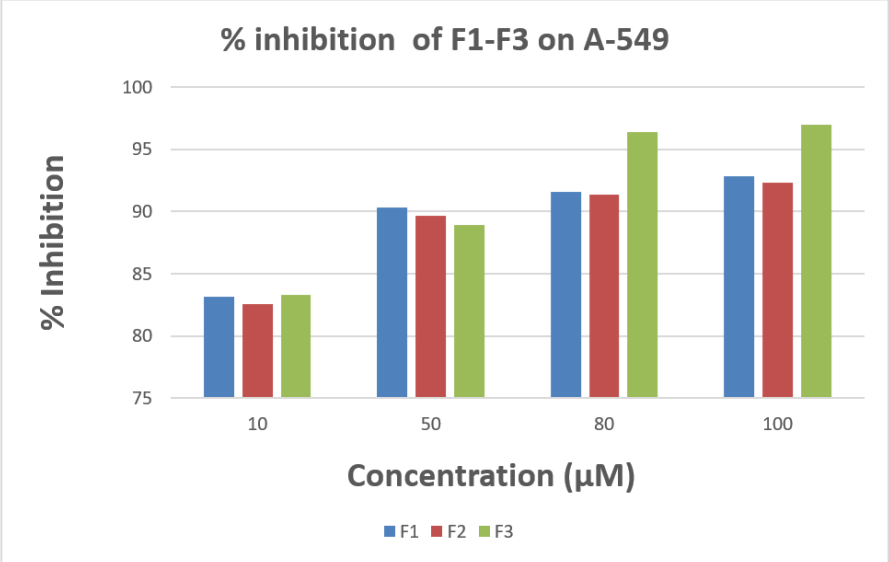

Figure 8: %inhibition of (F1-F3) on A-549

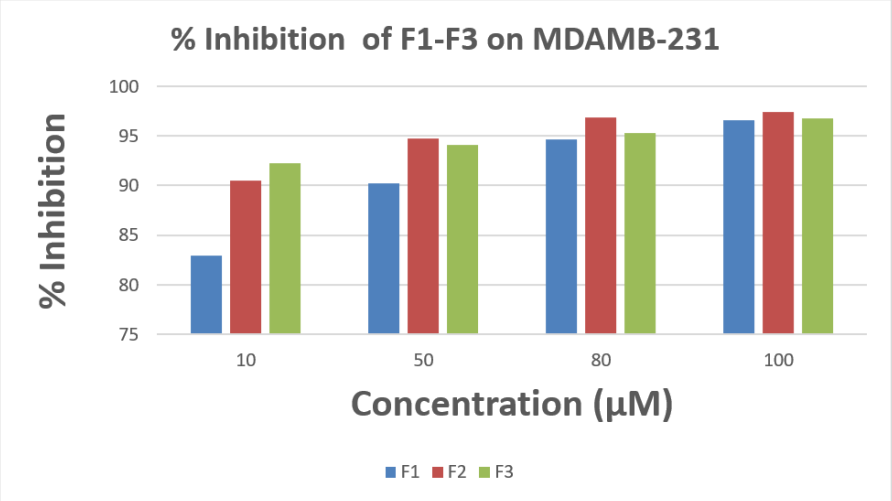

Figure 9: %inhibition of (F1-F3) on MDAMB-231

4. CONCLUSION

The cumulative percentage drug release of batch F4 to F6 is represented. The surface modification of optimum formulations was achieved with PEG 6000 duets its diverse characteristics such as hydrophilic nature, non-toxicity, unavailability of antigenic and immunogenic properties. The formulation of NPs (F3 and F6) exhibited slow release in comparison to the unmodified NPs. In vitro Cytotoxicity of formulation F4 to F6 central composite designed Polymer loaded nanoparticles (F4 to F6) and surface modified NPs (F4 to F6) prepared by nano-precipitation technique were studied. MTT assay was used to investigate the cytotoxicity of Polymer ratio and formulations on Human lung cancer lines-A549 by using concentrations range of 10-100 µg/mL. The cancer cells without treatment were used as control for estimation of percentage viability of different samples. Among all the formulations, polymer loaded all formulation showed the Cytotoxicity.

REFERENCES

1. Lotfipour, F., Abdollahi, S., Jelvehgari, M., Valizadeh, H., Hassan, M., & Milani, M. (2013). Study of antimicrobial effects of vancomycin loaded PLGA nanoparticles against enterococcus clinical isolates. *Drug research*, 348-352.
2. Mainardes, R. M., & Evangelista, R. C. (2005). PLGA nanoparticles containing praziquantel: effect of formulation variables on size distribution. *International journal of pharmaceutics*, *290*(1-2), 137-144.
3. Bandi, N. (2003). *Lung delivery of budesonide and its efficacy in a tumor model*. University of Nebraska Medical Center.
4. Fuliaş, A., Ledeţi, I., Vlase, G., Popoiu, C., Hegheş, A., Bilanin, M., ... & Moş, L. (2013). Thermal behaviour of procaine and benzocaine Part II: compatibility study with some pharmaceutical excipients used in solid dosage forms. *Chemistry Central Journal*, *7*, 1-10.

5. Darvesh, A. S., Carroll, R. T., Bishayee, A., Geldenhuys, W. J., & Van der Schyf, C. J. (2010). Oxidative stress and Alzheimer's disease: dietary polyphenols as potential therapeutic agents. *Expert review of neurotherapeutics*, *10*(5), 729-745.
6. Potdar, M. B., Jain, N. K., Patil, K. D., & Goyal, Y. S. (2022). Development of Carmustine Loaded PLGA-PEG Conjugates for Nose to Brain Targeting. *INDIAN JOURNAL OF PHARMACEUTICAL EDUCATION AND RESEARCH*, *56*(4), 1076-1082.
7. Wen, Z., Yan, Z., Hu, K., Pang, Z., Cheng, X., Guo, L., ... & Lai, R. (2011). Odorranalectin-conjugated nanoparticles: preparation, brain delivery and pharmacodynamic study on Parkinson's disease following intranasal administration. *Journal of controlled release*, *151*(2), 131-138.
8. Fazil, M., Md, S., Haque, S., Kumar, M., Baboota, S., kaur Sahni, J., & Ali, J. (2012). Development and evaluation of rivastigmine loaded chitosan nanoparticles for brain targeting. *European Journal of Pharmaceutical Sciences*, *47*(1), 6-15.
9. Md, S., Alhakamy, N. A., Aldawsari, H. M., & Asfour, H. Z. (2019). Neuroprotective and antioxidant effect of naringenin-loaded nanoparticles for nose-to-brain delivery. *Brain sciences*, *9*(10), 275.
10. Aktaş, Y., Andrieux, K., Alonso, M. J., Calvo, P., Gürsoy, R. N., Couvreur, P., & Çapan, Y. (2005). Preparation and in vitro evaluation of chitosan nanoparticles containing a caspase inhibitor. *International journal of pharmaceutics*, *298*(2), 378-383.
11. Tzeyung, A. S., Md, S., Bhattamisra, S. K., Madheswaran, T., Alhakamy, N. A., Aldawsari, H. M., & Radhakrishnan, A. K. (2019). Fabrication, optimization, and evaluation of rotigotine-loaded chitosan nanoparticles for nose-to-brain delivery. *Pharmaceutics*, *11*(1), 26.
12. Bari, N. K., Fazil, M., Hassan, M. Q., Haider, M. R., Gaba, B., Narang, J. K., ... & Ali, J. (2015). Brain delivery of buspirone hydrochloride chitosan nanoparticles for the treatment of general anxiety disorder. *International journal of biological macromolecules*, *81*, 49-59.
13. Kumar, M., Pandey, R. S., Patra, K. C., Jain, S. K., Soni, M. L., Dangi, J. S., & Madan, J. (2013). Evaluation of neuropeptide loaded trimethyl chitosan nanoparticles for nose to brain delivery. *International journal of biological macromolecules*, *61*, 189-195.
14. Trapani, A., De Giglio, E., Cafagna, D., Denora, N., Agrimi, G., Cassano, T., ... & Trapani, G. (2011). Characterization and evaluation of chitosan nanoparticles for dopamine brain delivery. *International journal of pharmaceutics*, *419*(1-2), 296-307.
15. Yu, S., Xu, X., Feng, J., Liu, M., & Hu, K. (2019). Chitosan and chitosan coating nanoparticles for the treatment of brain disease. *International journal of pharmaceutics*, *560*, 282-293.
16. Oza, G., Pandey, S., Mewada, A., Kalita, G., Sharon, M., Phata, J., ... & Sharon, M. (2012). Facile biosynthesis of gold nanoparticles exploiting optimum pH and temperature of fresh water algae Chlorella pyrenoidusa. *Adv Appl Sci Res*, *3*(3), 1405-1412.
17. Higuchi, T., & Connors, K. A. (1965). Advances in analytical chemistry and instrumentation. *Phase Solubility Stud.*, 117–212.
18. Kolodziejczyk, A. A., Kim, J. K., Svensson, V., Marioni, J. C., & Teichmann, S. A. (2015). The technology and biology of single-cell RNA sequencing. *Molecular cell*, *58*(4), 610-620.
19. Hoare, S. R., Fleck, B. A., Williams, J. P., & Grigoriadis, D. E. (2020). The importance of target binding kinetics for measuring target binding affinity in drug discovery: a case study from a CRF1 receptor antagonist program. *Drug Discovery Today*, *25*(1), 7-14.

Cite This Article: Mrugendra B. Potdar & Neetesh K. Jain (2023). Formulation and *In vitro* Cytotoxicity Studies of Avastin Nanoparticle Mediated Targeted Nose to Brain Drug Delivery System. *EAS J Pharm Pharmacol, 5*(5), 122-131.