Formulation & Development of Nanoparticles of 5-flurouracil for the treatment of Cancer.

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Abstract:

Cancer is the cause of the disease worldwide public health issue. Chemotherapy is the most widely used treatment for cancer; however its effectiveness is not ideal mostly because of nonselective toxicity. In the current studies nanoparticles prepared using 5-flurouracil for the treatment of cancer. Studies of Nanoparticles on different parameters like In-Vitro drug release, drug entrapment, particle size determination, blood level studies.

The concentration of the sample had an inverse relationship with its decline. The sample consists of FAC nanoparticles loaded with 5-FU. The percentage growth inhibition of the cell is shown. The findings indicated that a greater quantity of 5-FU hinders the development of cells. In addition, the cell viability decreases as the concentration of 5- FU increases. 5-FU is accessible either in its free form or contained within nanoparticles. The cytotoxicity of NPs formulations was observed to be higher within the concentration range of 10- 80 µg/ml, compared to plain 5-FU. The percentage growth inhibition of cell (A-549) was found to be higher with increasing concentrations of 5-FU, indicating that the cell growth was inhibited. The cytotoxicity of NPs formulations was o b se rve d to be higher w i t h i n the concentration range of µg/ml, compared to plain 5-FU. The improved 5-FU loaded FAC NPs were found to have a more potent inhibitory effect on A549 cells, indicating their cytotoxicity.

Keywords: Cancer, Nanoparticles, Drug entrapment, Blood level, Zeta Potential & Particle size, In-Vitro release,

Introduction: Cancer continues to be a significant cause of death and is a worldwide public health issue. Currently, chemotherapy is the most widely used treatment for cancer. However, its effectiveness is not ideal mostly because of the nonselective toxicity of the drugs used in chemotherapy. Therefore, the concept of cancer nanotechnology is proposed as a distinct method to combat cancer by using nanotechnology in the treatment of cancer [1].

Nano-carriers provide several benefits over traditional formulations when it comes to delivering bioactive chemicals. These advantages include the ability to encapsulate the agents inside the core of the nanocarrier or absorb them onto its surface. (1) By employing both passive and active targeting strategies, Nano carriers can ensure the safety and effectiveness of cancer treatment by altering the distribution of loaded drugs within the body(specifically, increasing drug concentration in tumor sites while minimizing accumulation in normal tissues). (2) Taking into account the biodegradability, pH, ion sensitivity, or temperature sensitivity of materials, Nano vehicles can be modified to release drugs in a controlled manner[2]. (3) Nano vehicles have the capability to load multiple agents through a rational design, enabling the implementation of combination therapy for cancer. Cancer is a malignant illness that has emerged as the leading cause of mortality

worldwide in recent times. Although extensive researches spanning many decades and substantial financing have enhanced our comprehension of basic processes such as carcinogenesis, the mortality rate attributed to cancer continues to stay elevated to this day. Cancer is a leading global source of sickness and infection. The World Health Organization has indicated that lung, colorectal, stomach, and breast cancer are the most often diagnosed types of cancer worldwide [3]. In addition to this, lung cancer is presently the leading cause of cancer and accounts for around 16.7% of diagnoses in males.

Material and Methodology

5-Fluorouracil (5-FU) was acquired as a complimentary sample from Neon Pharmaceuticals (MS, India). The Cellulose Acetate Phthalate (CAP) with a molecular weight of 6000 Daltons was obtained from Central Drug House (CDH) in Delhi, India. Folic acid (FA) was generously provided by

Himedia, a company based in Mumbai, India. The chemicals N- hydroxysuccinimide (NHS), dialysis membranes, 1-ethyl3-(3-dimethylaminopropyl) carbodiime hydrochloride (EDAC), Dicyclohexyl Carbodiimide (DCC), and Pluronic F-68 were acquired from Himedia laboratories in Mumbai, India. The compound adipic acid dihydrazide (ADH) was acquired from Sigma Aldrich. The acetone, isopropyl alcohol, and acetonitrile obtained from Merck Limited in Mumbai, India, as well as other chemicals utilized, are of investigative chemical quality and are used as purchased.

Method

In Vitro drug release

In vitro drug release depends upon the solvent system used in the manufacturing of the nanoparticle carrier, namely acetone and isopropyl alcohol, can disrupt the hydrogen bond of FA. This disruption promotes the reaction between the carboxyl group of FA and the amine group of adipic acid dihydrazide through carbodiimide conjugation. As a result, a cross linked core-shell micelle is formed, which has lower solubility and can facilitate sustained release.

Folic acid-ADH-CAP (FAC) Copolymer Amalgamation

Initially, 100 mg of FA was evenly distributed in 10 ml of distilled water. Then, 500 mg of EDAC was introduced into the FA dispersion while continuously stirring. EDAC activated the FA end groups and facilitated strong interaction with other molecules. Subsequently, 500 mg of adipic acid dihydrazide was added to the activated FA dispersion. “The reaction was allowed to proceed for 6 hours at room temperature. The CAP was dissolved in a solvent system consisting of acetone (10ml). Then, dicyclohexyl carbodiimide (100mg) and N-hydroxysuccinimide (50mg) were added to the solution while stirring continuously for a period of” 12 hours. After the formation of CAP dispersion, it was added drop by drop at regular intervals into the FA-ADH solution and stirred for 6 hours to ensure proper and uniform interaction between the amine groups of ADH and the carboxyl group of CAP, resulting in the formation of amide bonds through carbodiimide conjugation. This demonstrates that FA-ADH binds with the polymer CAP. The copolymer, known as FAC, is a compound consisting of two or more different monomers that are chemically bonded together.

The collected sample was subjected to vacuum drying and then authenticated using 1H- NMR (Bruker DRX, USA at 400 MHz) and FTIR (IR Tracer-100, Shimadzu) spectroscopic techniques [4].

Zeta Potential Determination, Particle Size analysis

The particle size assessment of FAC nanoparticles was conducted using a particle size analyzer, Zeta Sizer Nano ZS (Malvern Instruments) or comparable zeta potential instrument is used for

detection of the Zeta Potential. Folded capillary cell (referred to as zeta cells, polycarbonate cell with gold-plated electrodes; Malvern Instruments, DTS1060C or equivalent) Caps for zeta cells (2 per cell).

Zeta Potential Transfer Standard (Carboxylated polystyrene latex dispersed in pH 9.22 buffer; Malvern Instruments, DTS0050) 10 mM NaCl. The surface characteristics like shape, size and texture was examined by using atomic force microscopic technique (SPM-9500, Shimadzu).

Measurement of Drug entrapment proficiency

The entrapment efficiency of the drug nanoparticles was influenced by the concentration of surfactant and polymers, as demonstrated in the table. By increasing the amount of cellulose acetate phthalate (a type of polymer) from 10 mg to 30 mg, the size of FAC particles increases within the range of 84. The wavelength was reduced from 208nm to 93nm, resulting in a fall in entrapment efficiency of the medication from 93% to 72. The SLN dispersion was centrifuged at 5,000 rpm for 30 minutes. The quantity of free drug in the dispersion medium was determined. The amount of miconazole in the aqueous phase was recorded at 274 nm.

Encapsulation efficiency was calculated as follows: Encapsulation

efficiency (%)=100−(Fs/Ts×100)(1)

Where Fs is the free soluble drug and Ts is the initial amount of drug added during the preparation of MN-SLNs.

Blood level study

The blood level study of loaded FAC NPs preparations was implemented on the animal (albino rats) to establish the expediency of delivering 5-FU into the blood by intravenous (IV) administration.

Results

In-vitro drug release pattern

The graph (Fig. 7.6, Table 7.5) demonstrates the extended and continuous release characteristics of the medication from the nanoparticle system. The drug release graph depicts the controlled and sustained release of 5-FU from both the FAC system and the simple polymeric (CAP) nanoparticulate system. The FAC nanoparticles exhibited sustained release of 5FU for up to 48 hours, with a release rate of 98.52%. In contrast, normal CAP NPs released 97.5% of 5-FU within 12 hours. This is because the solvent system used in the manufacturing of the nanoparticle carrier, namely acetone and isopropyl alcohol, can disrupt the hydrogen bond of FA. This disruption promotes the reaction between the carboxyl group of FA and the amine group of adipic acid dihydrazide through carbodiimide conjugation. As a result, a cross linked coreshell micelle is formed, which has lower solubility and can facilitate sustained release [5-7].

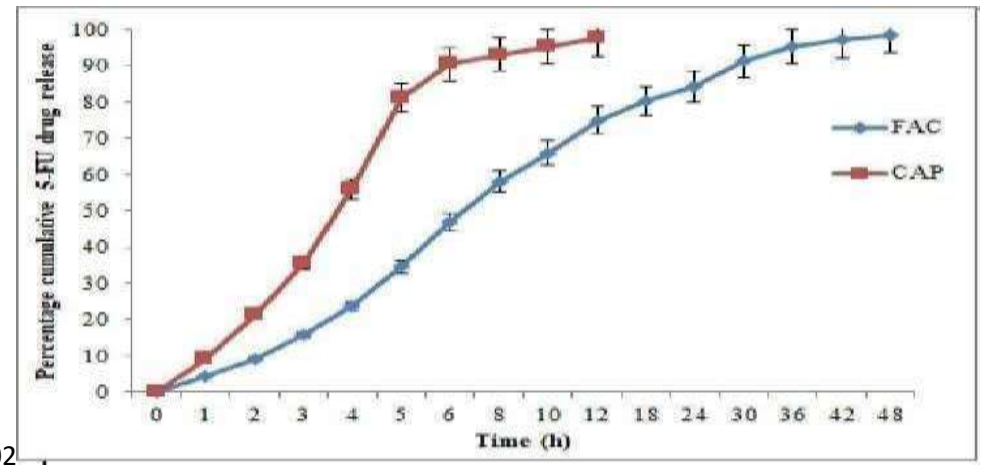

Figure 1 Percentage cumulative 5-FU release of FAC and CAP NPs

Original

Table 1 Cumulative percentage drug release (5-FU) from FAC and CAP nanoparticle

S. No.	Hrs.	Cumulative percentage drug release of FAC NPs	Cumulative percentage drug release of CAP NPs
1	1	4.2±0.12%	16.3±0.51%
2	2	11.3±0.20%	31.5±0.60%
3	3	23.2±0.5%	46.8±0.75%
4	4	32.5±0.67%	63.9±0.80%
5	5	45.9±0.75%	75.9±0.95%
6	6	56.6±0.88%	84.3±0.98%
7	7	64.2±1.1%	92.7±1.15%
8	8	70.7±1.12%	97. ±1.26%
9	9	75.2±1.15%	-
10	10	80.1±1.25%	-
11	11	85.2±1.25%	-
12	12	90.3±1.3%	-
13	24	94.3±1.5%	-
14	32	96.21±1.52%	-

Hemolytic toxicity study

The hemolytic toxicity investigation was conducted to determine the hemotoxic effect of the designed FA attached cellulose acetate phthalate nanoparticle and plain cellulose acetate phthalate nanoparticles. The unmodified 5-FU, 5-FU encapsulated in FAC NPs, and 5-FU encapsulated in FAC NPs Cationic antimicrobial peptides (CAP NPs) have demonstrated hemolytic toxicity levels of up to 29.15±1.12%, 4.60±0.5%. The individual percentage of

8.65±1.05% was observed in distilled water according to Table 7.6. The formulations of plain 5- FU and 5-FU loaded nanoparticles had 0.1μM equivalents of 5-FU. The efficacy of the 5-FU nanoparticle formulation was assessed based on the drug content. The delayed release of encapsulated drug molecules in the nanoparticles resulted in a reduction in hemolytic toxicity. The results from the hemolytic toxicity investigation showed that the 5-FU containing FAC NPs had lower hemotoxicity compared to the 5-FU loaded CAP NPs. This could be attributed to the hydrophilic properties of FA, which contribute to the development of a hemocompatible system. The suppression of drug-induced hemotoxicity can be associated with prior investigations on nanoparticle [8].

Table 2 Absorbance of hemolytic standard at 540nm.

S. No.	Hemolytic Standard	Absorbance at 540nm
1	Hemolytic Standard of distilled water	0.1998

Table 3 Data of absorbance of 10% hematocrit solution at 540nm.

S. No.	10% hematocrit solution	Absorbance at 540nm	Percentage cumulative drug release
1	10% hematocrit solution of distilled water with FAC NPs	0.1910	4.60%
2	10% hematocrit solution of distilled water with CAP NPs	0.1825	8.65%

Blood level study

A study was conducted to measure the concentration of the drug in the blood plasma after giving albino rats an intravenous "dose of 5-FU laden FAC NPs and a 5-FU solution. Three distinct cohorts of albino rats were established, with each cohort consisting of six rats. The animal subjects were divided into two groups. The first group received a simple solution of 5-FU" and FAC NPs through intravenous administration. The second group also received the same treatment. The third group, which served as the control, was not given any therapy and instead received a healthy diet. Following the delivery of samples, about 0.1 ml of blood was taken from the retro-orbital plexus of albino rats at 1, 2, 4, 6, and 8-hour intervals for a total of 24 hours. The blood samples were centrifuged at 5000 rpm for 15 minutes to separate the supernatant (serum). The serum was then deproteinized by adding an equal volume of acetonitrile (1ml/ml of serum).

Ultimately, the drug content was the obtained samples were examined utilizing High Performance Liquid Chromatography (HPLC) to analyze the supernatants [9].

Zeta Potential Determination, Particle Size analysis and Drug Entrapment Proficiency The particle size assessment of FAC nanoparticles was conducted using a particle size analyzer, revealing a particle size of FAC nanoparticles of 84±1.10 nm. The particle size distribution ranges from 84±1.10nm to 208±2.10nm due to the aggregation of particles.

Based on the data presented in Table 4, increasing the amount of cellulose acetate phthalate (CAP-polymer) from 10mg to 30mg resulted in an increase in particle size from the range of 84±1.10nm to 208±2.10nm. The particle size range derived from CAP NPs ranged from 105±1.50 to 287±2.25nm, as shown in Table 5. The polydispersity index (PDI) of FAC nanoparticles was determined to be 0.073 ± 0.05, while the PDI value of CAP nanoparticles was measured to be

0.079 ± 0.10. The encapsulation efficiency of prepared FAC nanoparticles and CAP nanoparticles was found to be 93.65±1.15% and 90.50±1.15% respectively. The zeta potential of FAC nanoparticles was measured to be - 13.1 mV, whereas for CAP nanoparticles it was discovered to be -5.98 mV (Table 6).

Table 4 Ingredients and concentration using in the formulation of FAC nanoparticles.

S. No.	Drug: Polymer ratio (mg)	Internal phase			External phase		% Entrapment efficiency	Particle size (nm)
		5-FU (mg)	FAC (mg)	Acetone (ml)	Pluronic F68 (mg)	Water (ml)		
S1	30:10	30	10	20	250	25	74.50±1.50	197±1.15
S2	30:20	30	20	20	250	25	80.11±1.15	139±0.50
S3	30:30	30	30	20	250	25	93.65±1.15	84±1.10
S4	30:10	30	10	20	500	25	72.61±0.75	208±2.10
S5	30:20	30	20	20	500	25	78.32±0.75	165±1.15
S6	30:30	30	30	20	500	25	85.45±0.50	117±1.50

S: Sample, 5-FU: 5-Fluorouracil

Table 5 Ingredients and concentration using in the formulation of CAP nanoparticles.

S. No.	Drug: Polymer ratio (mg)	Internal phase			External phase		% Entrapment efficiency	Particle size(nm)
		5-FU (mg)	CAP (mg)	Acetone (ml)	Pluronic F-68(mg)	Water (ml)		
S1	30:10	30	10	20	250	25	64.25±1.50	258±0.50
S2	30:20	30	20	20	250	25	74.50±0.50	199±0.15
S3	30:30	30	30	20	250	25	90.50±1.15	105±1.50
S4	30:10	30	10	20	500	25	60.15±0.50	287±2.25
S5	30:20	30	20	20	500	25	68.21±1.75	235±1.17
S6	30:30	30	30	20	500	25	80.20±0.25	142±1.15

S: Sample, 5-FU: 5-Fluorouracil

Table 6 Optimum particle size &entrapment efficiency of FAC and CAP nanoparticles.

S. No.	Formulations	Entrapment efficiency	Particle size (nm)	Polydispersity Index	Zeta Potential
1.	FAC NPs	93.65±1.15	84±1.10	0.0732±0.05	-13.1 mV
2.	CAP NPs	90.50±1.15	105±1.50	0.079±0.10	-5.98 mV

Blood Level

Table 7. Calibration curve 5-FU in serum.

S. No	Concentration (µg/ml)	Absorbance (at 265nm)	Equation
1.	2	0.0539	y = 0.054x - 0.000 R^2 = 1
2.	4	0.1065	
3.	6	0.1619	
4.	8	0.2141	
5.	10	0.2688	
6.	12	0.3245	
7.	14	0.3769	
8.	16	0.4323	
9.	18	0.4849	
10.	20	0.5381	

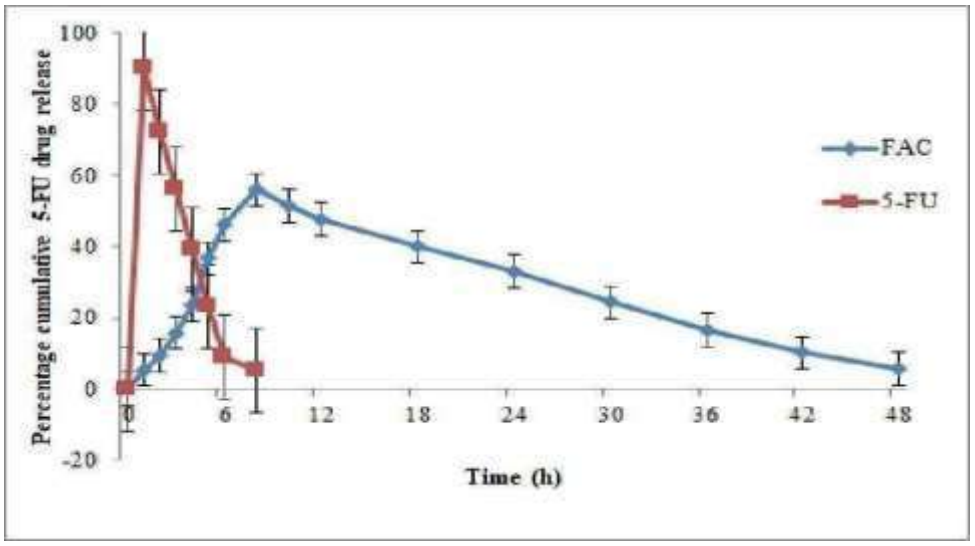

Figure 2 Concentration of 5-FU in serum after IV administration of Plain 5-FU and 5- FU loaded FAC NPs formulations in albino rats.

Statistical analysis

The final research results were presented as the mean ± standard deviation (SD). This evaluation was conducted using a ttest. The significance of $P < 0.05$ was particularly noteworthy. The procedures were executed three times.

Conclusions

The concentration of the sample had an inverse relationship with its decline. The sample consists of FAC nanoparticles loaded with 5-FU. The percentage growth inhibition of the cell is shown. The findings indicated that a greater quantity of 5-FU hinders the development of cells. In addition, the cell viability decreases as the concentration of 5- FU increases. 5FU is accessible either in its free form or contained within nanoparticles. The cytotoxicity of NPs formulations was observed to be higher within the concentration range of 10- 80 µg/ml, compared to plain 5-FU. The percentage growth inhibition of cell (A-549) was found to be higher with increasing concentrations of 5-FU, indicating that the cell growth was inhibited. The cytotoxicity of NPs formulations was observed to be higher w i t h i n the concentration range of 10-80 µg/ml, compared to plain 5-FU. The improved 5-FU loaded FAC NPs were found to have a more potent inhibitory effect on A549 cells, indicating their cytotoxicity.

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