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LESS TOXIC NANOPARTICLES OF PLATINUM BASED ANTI-CANCER DRUG

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ABSTRACT: Objectives: The objective of this research was dose reduction and toxicity reduction. Dose reduction: various anti-cancer drugs are very costly, and their high dose increases cost. If bioavailability is enhanced, the dose shall be reduced thereby; the cost will also decrease. Dose reduction will also reduce toxicity, and the preparation will become more tolerable. **Method:** In this study, the nanoparticles were developed by using a modified method with the drug cisplatin, which was later evaluated for stability, toxicity, and therapeutic efficacy using various *in-vitro* and *in-vivo* techniques like SRB assay. **Results:** The resulting dosage form found to be more affected and less toxic than the marketed preparation of drug cisplatin, which is i.v. injection. As cisplatin is a platinum compound and has the biggest drawback of the toxic side effects, which is making the chemotherapy less acceptable but with the preparation of nanoparticles side effects were minimized without harming the therapeutic efficacy. Anti-cancer agents have several adverse side effects and high levels of toxicity, *e.g.,* Hair loss due to the effects on hair follicles, anemia, immune system impairment, and clotting problems, reduction in the number of red cells, white cells, and platelets. **Conclusion:** The nanoparticles were prepared using a new generation polymer, namely EUDRAGIT. The method was self-modified for the preparation of these nanoparticles. Process optimization and validation were done before the final nanoparticles were obtained. The nanoparticles obtained were smooth and almost round as elicited by SEM and stable more than a year as concluded from stabilities studies.

INTRODUCTION: Cancer is a disease characterized by the uncontrollable growth of cells [8, 38]. There are more than 100 types of cancer known which are classified by the type of cell they initially affect. It is a result of the cell that grows uncontrollably and does not die or forgets to die [22].

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Or can be said the absence of apoptosis of the grown cell leads to form a mass of abnormally grown cells that harms the body [13]. Cancer is the leading cause of morbidity and mortality worldwide. In the last few years, there is an enormous increase in new cancer cases worldwide. Cancer can be treated with chemotherapy, radiation therapy, hormone therapy, immuno- or gene therapy and surgery [35].

In chemotherapy, the drug or the combination of drugs are selected depending upon the type of cancer. Conventional chemotherapy is only good for the treatment of cancer that has spread or

metastasized, because the drug travels throughout the body without recognizing the cancer site [1, 31, 39]. In some form of lymphomas and leukemia, it is the only option as surgery or radiation is not much feasible. Conventional drug delivery system cannot differentiate between the normal and cancerous cells and thus affect the normal cells adversely [2, 45, 51]. The chemotherapy has several side effects like nausea, hair loss, fatigue vomiting, *etc*. [23, 26, 39]. Platinum-based drugs are widely used as anticancer agents in chemotherapy, particularly against ovarian and lung cancer. The most commonly used platinum-based drug, cis-diamminedichloro-platinum(II) (cisplatin), it is an alkylating agent with cytotoxic activity in cancer. The antitumor properties of cisplatin are attributed to its DNA cross-linking activities. Common problems associated with the clinical use of cisplatin are cumulative toxicities like nephrotoxicity, ototoxicity, and peripheral neuropathy [6, 44, 51]. In addition to the serious systemic toxicities, rapid blood clearance, and inherent or treatment-induced resistant tumor cell subpopulations limit the therapeutic efficacy of cisplatin [15, 47]. The idea that can be used to overcome the side effects of platinum drugs is to form a more novel drug delivery system like nanoparticulated drug delivery systems.

Nanoparticles are usually smaller than several hundred nanometers in size, comparable to large biological molecules such as enzymes, receptors, of size about 100 to 10,000 times smaller than human cells [2, 39]. These nanoparticles can offer unprecedented interactions with biomolecules both on the surface and inside the body cells, which may bring revolution in cancer diagnosis and treatment [21, 33, 49]. At the nanometric scale, the physicochemical and biological properties of materials differ fundamentally from their corresponding bulk counterpart because of the size-dependent quantum effect. It is known that nanoparticles may possess extraordinary, often tunable properties dramatically different from their bulk material; consequently, there is an enormous demand for tailor-made functional nanoparticle systems. Inorganic, organic, or hybrid nanoparticular materials are used in various application fields such as medicine, pharmaceutics, analytics, catalysis, coatings, and several others. The production of such nanoparticle systems requires specific characteristics of the materials used. Essentially, the various nanoparticle preparation techniques can be sorted into two general categories. The first category involves the in situ reactive syntheses of nanoparticles, starting from solubilized small molecule precursors (*e.g.*, preparation of gold nanoparticles, emulsion polymerization techniques). In the second category, the shaping of the bulk material into nanostructures (*e.g.*, nanoprecipitation, emulsion/solvent diffusion technique, spray drying, salting out, and milling processes) yields nanoparticles based on low as well as high molar mass compounds.

In this context, polymeric nanoparticles represent particularly rich opportunities to tune and control the outcome of the nanoparticle materials, since they can be processed with various functionalities as well as characteristics and can thus cover broad application fields [12, 16, 17, 18]. A polymer, natural or synthetic is a substance that is combined with a drug or other active agent to release the drug in a pre-designed manner [1, 37]. The development of Novel drug delivery system has been made possible by the various compatible polymers to modify the release pattern of drug [5, 26]. The basic purpose of controlled drug release is to achieve more effective therapies by eliminating the potential for both under- and overdosing. Other advantages are the maintenance of drug concentration within the desired range, fewer administrations, optimal drug use, and increased patient compliance [28].

MATERIALS AND METHODS:

Materials:

Procurement of Raw Materials: EUDRAGIT RS100, was obtained as a gift sample from Evonik Degussa India, Pvt. Ltd., Mumbai. PVA from Ranbaxy, Mumbai. Analytical grade Lab reagents and other solvents obtained from Qualigen Fine Chemicals, Mumbai, in small amounts.

Procurement of Drug: Drug used in formulating nanoparticles, *i.e.*, cisplatin was procured from Khandelwal laboratories Pvt. Ltd., Rudrapur as a small sample for individual research purpose.

Methods:

Preparation of Nanoparticles:

Placebo Eudragit RS100 nanoparticles (Blank): Previously, these types of nanoparticles were

prepared using the reported method, *i.e.*, by emulsion solvent evaporation method [28]. But in later stages after determining various properties of the polymer and the solubility of the drug and the polymer, placebo nanoparticles were prepared by a self-modified method as follows:- 0.5 g Eudragit RS100 was dissolved in 8 ml acetone and 1 ml DMSO. Then 2 ml of methanol was added to 25 ml water. Tween 80 2% v/v was added to this solution with stirring on magnetic stirrer having 500 rpm. The aqueous phase was kept under ultra probe sonication with 80% power for 10-15 sec for vanish the tween 80 bubbles. Then this solution was kept over an ice bath, sonicated for 5 min with the addition of organic phase with the syringe at a slow rate of approx 3 ml per min. after resting for 5-sec sonication again done for 2-3 min. The solution was sonicated several times with interval over an ice bath for about 30 min. Then the resultant solution was kept for a few hours over magnetic stirrer to ensure complete evaporation of organic solvents. The suspension was prepared in the range of nanosuspension was cryo-centrifuged for 45 min at about 15000 rpm. The supernatant was discarded, and the sediment was washed twice with distilled water and kept for freeze drying at -40 ºC for about several hours. Then obtained dried powder was kept in refrigeration for further experimentation.

Cisplatin Nanoparticles: Nanoparticles were prepared after determining various properties of the polymer and the solubility of the drug and the polymers. Nanoparticles were prepared by using self-modified emulsion solvent evaporation method [28] varying drug: polymer ratio. The nanoparticles were prepared by fixing the amount of drug to be incorporated to be constant at 10 mg. 0.25 g, 0.5 g, 1.0 g Eudragit RS100 has dissolved 4 ml, 8 ml, 16 ml of acetone respectively. 10 mg Cisplatin dissolved in 1 ml DMSO and was also added to this solution. The solution was kept in an airtight flask for 15 min. Then 2ml of methanol was added to 25 ml water. And Tween 80 2% v/v was added to this solution with stirring on magnetic stirrer having 500 rpm. The aqueous phase was kept under ultra probe sonication with 80% power for 10-15 sec for vanish the tween 80 bubbles. Then this solution was kept over an ice bath, sonicated for 5 min with the addition of organic phase with the syringe at a slow rate of approx 3 ml per min., after resting for 5-sec sonication again done for 2-3 min. The solution was sonicated several times with interval over an ice bath for about 30 min. Then the resultant solution was kept for a few hours over magnetic stirrer to ensure complete evaporation of organic solvents. The suspension was prepared in the range of nanosuspension was cryo-centrifuged for 45 min at about 15000 rpm. The supernatant was discarded, and the sediment was washed twice with distilled water and kept for freeze drying at -40 ºC for about several hours. Then obtained dried powder was kept in refrigeration for further experimentation [2, 5].

Evaluation of Nanoparticles:

Particle Size Distribution and Zeta Potential: The particle size of various placebo nanoparticles and drug-loaded nanoparticles were measured by photon correlation spectroscopy and laser Doppler using a zetasizer. The refrigerated formulation was suspended in deionized water by using sonicator. This suspension used for particle size distribution. The same suspension was used for zetasizer also [29, 40].

Surface Characteristics: The surface characteristics of particles were studied by performing the morphological examination of the nanoparticles using scanning electron microscopy by using 1 drop of redispersed freeze dried particles prepared by sonicating it with deionized water. The drop was placed over copper grid/stud and dried under vacuum. It is observed under SEM without staining [42, 46].

Physical Characteristics: The physical characteristics were determined by visual examination [42, 46].

Size Distribution and Zeta Potential: The same method used as in case of placebo nanoparticles but keeping in mind to avoid the usage of metals reactive than platinum like aluminum as our drug contains platinum which may replace with aluminum.

Surface Characteristics: The surface characteristics of particles were studied by performing the morphological examination of the nanoparticles using scanning electron microscopy by using 1 drop of redispersed freeze dried particles prepared by sonicating it with deionized water. The drop was placed over copper grid/stud and dried

under vacuum. It is observed under SEM without staining [42, 46].

Determination of Drug Content and Entrapment Efficiency: [27, 39]

Drug Content: The drug content was determined using a nanoparticle dissolution method. The known amount nanoparticles were dissolved in a 1:1 ratio of acetone and water for Eudragit RS100 formulations.

For the determination of drug entrapment, the amount of drug present in the clear supernatant after centrifugation (w) was determined by UV-spectrophotometry. A standard calibration curve of concentration versus absorbance was plotted for this purpose. The amount of drug in the supernatant was then subtracted from the total amount of drug added during the process (W). In effect, (W-w) will give the amount of drug entrapped in the nanoparticle. Then, the percentage of entrapment is given by:

$$\text{Entrapment efficiency} = (W - w) \times 100 / W$$ [32]

Drug and Polymer Compatibility Study: For compatibility, the physical mixtures were analyzed with FT-IR.

IR Studies: The physical mixtures or drug and the polymers, pure drugs and pure polymers were analyzed by pressing pellet method. A minimum amount of sample was thoroughly blended with the adequate quantity of IR grade KBr. This mix was then made into KBr pellets by using a hydraulic press. The samples then analyzed in double beam IR spectrometer using KBr film as a negative control (blank). The scanning range was 4000^{-1}-$400^{-1,}$ and resolution was 16 cm. The spectra of the physical mixture and the pure drug and polymer were interpreted for the compatibility [4, 7, 11].

Stability Study: Stability studies generate the information on which proposal for the shelf life of formulation and recommended storage is based. The formulation was tested for stability by storing them at 4 ºC ±1 in refrigerators, 25 ºC & 40 ºC (±2 ºC) with the relative humidity of 70% for three months. After 1 month, 2 months, and 3 months, they were evaluated [4, 7].

***In-vitro* Release Study:** The *in-vitro* drug release was done in glass bottles containing 100 ml of phosphate buffer saline/0.1N HCl. To these bottles, 0.5 mg of nanoparticles already freeze dried was added and kept upon a magnetic stirrer.

After a specified interval, 2 ml sample was collected and centrifuged at an rpm 15000 for 30 min at 4 ºC. The supernatant collected and analyzed by UV Vis spectrophotometer. The precipitate was re-suspended in 2 ml fresh buffer and transferred back to the glass bottle. The study carried out in triplicate [41, 43, 54].

Release Kinetics: To understand the mechanism and kinetics of drug release, the drug release data of the *in-vitro* dissolution study was analyzed with various kinetic models like zero order, first order, Higuchi's, Korsmeyer-peppa's and coefficient of correlation (R^2) values. The data obtained from release studies were fitted to various kinetic equations [32].

***In-vivo/ex-vivo* Evaluation:** Anticancer activity in comparison to marketed preparation is done using SRB assay on ovarian SK-OV-3 and lung cancer cell lines A-549.

Toxicity Studies: Acute toxicity: the formulation will be administered in albino rats at prescribed dosage for cancer. The animals will be observed up to 4 days for mortality or any adverse reactions like increased or decreased motor activity, tremors, convulsions, Straub reaction, spasticity, loss of righting reflex, sedation, hypnosis, lacrimation, salivation, depression, or stimulation of respiration, prioritize, occurrence of sore/ulcers/rashes and the results will be compared with a marketed preparations.

Sub Acute Toxicity: the formulation will be administered in albino rats 1 time/ 2 times the test dose; they will be observed up to 40 days for mortality, or any other adverse reactions. The results will be compared with the marketed preparations. The animals will be administered the dose once every 10 days.

Safety Studies: the safety studies of the prepared nanoparticles were done by biochemical and hematological parameters of albino rats. The therapeutic dose for cancer is calculated for albino rats and administered through i.p. route. The calculated dose of prepared nanoparticles and the

marketed formulation was administered to the animals for comparison of toxicity. The various parameters like weight, water intake, food intake, SGOT, SGPT, blood urea, creatinine, and hematological parameters, *i.e.,* complete blood count was monitored till signs of recovery [2, 4, 18].

RESULTS AND DISCUSSION:

Formulation Development:

Preparation of Placebo Nanoparticles: The particles prepared from the Resomer polymers are gritty, sticky, collagen-like, rough, hard, dry, and translucent with a yellowish tinge. Whereas the Eudragit nanoparticles are also sticky, rough, and hard but having a blue translucence.

Preparation of Drug Loaded Nanoparticles: The nanoparticles prepared by different polymers and drugs were illustrated in **Table 9**.

Determination of Drug Content or Loading Efficiency: The % drug loaded or drug content was calculated as per the above-defined formula:

$$\% \text{ Drug content} = (W\text{-}w) \times 100 / W$$

The results found illustrated in **Table 10**:

TABLE 1: FORMULATION NOMENCLATURE

Name of the polymer	Name of the drug	Name of the nanoparticles	Name and amount of drug	Amount of polymer
Eudragit RS100 nanoparticles	Cisplatin	RS1	10mg	1g
	Cisplatin	RS2	10mg	0.5g
	Cisplatin	RS3	10mg	0.25g

TABLE 2: % DRUG CONTENT

Name of the formulation	Amount of drug in the supernatant (w)	Entrapment efficiency
RS3	0.7008 mcg	99.99%
RS2	0.6293 mcg	99.99%
RS1	0.9749 mcg	99.99%

Characterization of Nanoparticles:

Particle Size and Distribution: The particle size distribution and zeta potential is as follows in **Table 3**.

TABLE 3: DRUG CONTENT, ENTRAPMENT EFFICIENCY, PARTICLE SIZE AND ZETA POTENTIAL FOR EUDRAGIT RS100 AND CISPLATIN NANOPARTICLES *i.e.* RS1, RS2 AND RS3

Parameters	Size of nanoparticles	Zeta potential in mV	Amount of drug in supernatant (W)	Entrapment efficiency	Drug content
RS1	492 nm ± 2	-0.9 ± 1.2	0.5488 mcg	99.99%	88.5% ± 1.27
RS2	427 nm ± 22	-1.7 ± 1.2	0.2932 mcg	99.99%	79% ± 0.8
RS3	526 nm ± 20	-1.2 ± 0.8	0.1578 mcg	99.99%	84.42% ± 0.67

(n=3; mean ± s.d.)

Surface Characteristics: The surface morphology was analyzed by visual inspection of the photographs of SEM. Few Photographs are shown here the rest will be submitted with the final copy. The photographs of SEM are present in power point presentation.

Drug and Polymer Compatibility Study:

IR Studies: The results of IR studies confirm that there is no noticeable shifting as well as no less of functional peaks between the spectra of drug, polymer & drug-polymer mixture.

TABLE 4: THE % DRUG RELEASE DATA FOR RS1

Time	Amount of the drug released (in mcg)	% drug release = (drug release /amount of entrapped) × 100
1 h	0.124	0.012
2 h	1.317	0.131
5 h	53.68	5.368
22 h	395.88	39.59
26 h	598.7	59.87
28 h	641.98	64.19
46 h	810.699	81.07
50 h	847.74	84.77
52 h	872.43	87.24
54 h	910.93	91.09
55 h	973.212	97.32

***In-vitro* Release Study:**

Release Kinetics: The kinetics studies revealed that the release rate for the drug cisplatin through eudragit RS100 nanoparticles was second order type as evidenced by the graph shown in **Table 16**. The kinetics was second order type after the period 5 h. However, time increased the concentration of release drug kept on increasing for formulations, as shown in **Table 17**.

TABLE 5: CALCULATED DATA WITH THE CORRESPONDING GRAPH

Time	% Concentration released for RS1
1 h	0.012
2 h	0.131
5 h	5.368
22 h	39.59
26 h	59.87
28 h	64.19
46 h	81.07
50 h	84.77
52 h	87.24
54 h	91.09
55 h	97.32

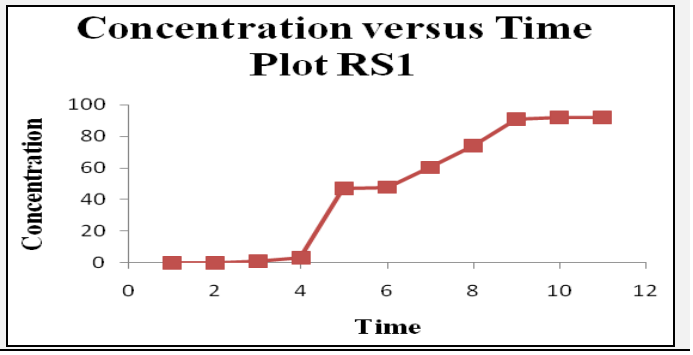
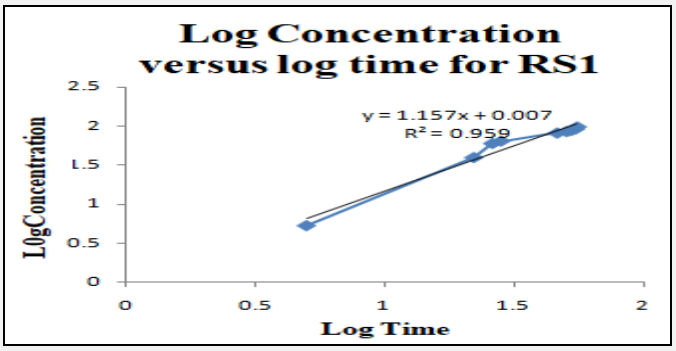


TABLE 6: LOG VALUES TO DETERMINE ORDER OF REACTION

Log Time	Log conc. of RS1
0	-1.92
0.301	-0.8827
0.698	0.7298
1.342	1.598
1.414	1.777
1.447	1.807
1.663	1.9088
1.699	1.928
1.716	1.94
1.732	1.959
1.74	1.988



Stability Study: The results of the stability studies suggest that the formulation was stable over one year at refrigeration and room temperature with the relative humidity of 75%. The nanoparticles are stored with and without redispersing in double distilled water. There were no significant changes in shape, particle size, viscosity, sedimentation, and drug content in any condition.

Sedimentation and Redispersity: Eudragit formulation, RS1, RS2, RS3 shows no evidence of any change in turbidity. Turbidity was 50, 30, and 20 NTU respectively, hence the formulation was found to be stable, and no sedimentation of suspended particles could be observed up to 1yr.

Viscosity: The viscosity of RS1 is 0.877 cp ±.05 at room temperature and 0.996 at refrigeration. The viscosity of RS2 is 0.762 cp ±.05 at room temperature and 0.952 cp at refrigeration. The viscosity of RS3 0.845 cp ±.05 at room temperature and 1.07 cp at refrigeration, which is not affected and remain the same throughout the shelf life.

Particle Size and Drug Content: The tables with the corresponding graph depicts the results of stability studies. The particle size affected only in refrigerated temperature there was no change in size in formulation stored at room temperature for any prepared formulation.

Hence, the results suggest all the formulations were stable on storage at refrigeration and room temperature (25 ºC ± 5). All the formulations when suspended in double distilled water, showed appreciable stability at both the storage temperature even in liquid dosage form without adding any stabilizers. As polymer Eudragit is not stable at room temperature, so it is suggested that the formulations of Eudragit RS 100 containing nanoparticles be stored at refrigeration after lyophilization.

TABLE 7: STABILITY STUDIES DATA FOR RS1 IN DOUBLE DISTILLED WATER (NANOSUSPENSION)

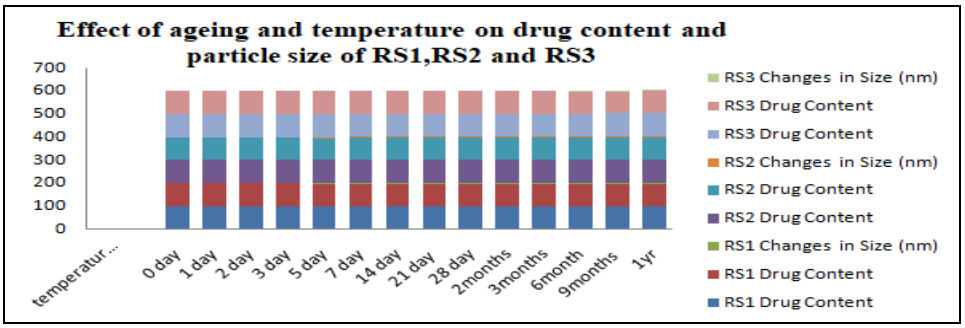
Period	Entrapment efficiency	Viscosity	Sedimentation	Redispersibility	Changes in size
	2-8 °C 25 °C (± 2 °C)				
0 day	99.9902 ± 0.07				0 nm
1 day	99.9902 ± 0.09				0 nm
2 day	99.9902 ± 0.02	0.877 cp ±.05 at room	No evidence of any	Not required as no	0 nm
3 day	99.9902 ± 0.1	temperature and 0.996	change in turbidity	sedimentation seen	0 nm
5 day	99.9902 ± 0.1	at refrigeration	hence no		+0.2 nm
7 day	99.9902 ± 0.005	throughout the shelf	sedimentation.		+0.5 nm
14 day	99.9895 ± 0.09	life	Turbidity was 50 ±		+0.6nm
21 day	99.9892 ± 0.2		5 NTU		+0.6nm
28 day	99.9888 ± 0.08				+0.6nm
2 month	99.998 ± 0.02				+0.8nm
3 month	99.998 ± 0.04				+0.9nm
6 month	99.9978 ± 0.07				+1.0nm
9 month	99.9976 ± 0.04				+1.6nm
1yr	99.9975 ± 0.1				+2.3nm

TABLE 8: STABILITY STUDIES DATA FOR RS2 IN DOUBLE DISTILLED WATER (NANOSUSPENSION)

Period	Drug content	Viscosity	Sedimentation	Redispersibility	Changes in size
	2-8 °C 25 °C (± 2 °C)				
0 day	99.9930 ± 0.09				0 nm
1 day	99.9930 ± 0.061				0 nm
2 day	99.9930 ± 0.066	0.762 cp ±.05 at room	No evidence of any	Not required as no	0 nm
3 day	99.9930 ± 0.097	temperature and 0.952	change in turbidity	sedimentation seen	0 nm
5 day	99.9930 ± 0.6	cp at refrigeration	hence no		+0.05 nm
7 day	99.9930 ± .0.1	throughout the shelf	sedimentation.		+0.05nm
14 day	99.99300 ± 0.3	life	Turbidity was 30 ±		+0.05nm
21 day	99.9929 ± 0.5		10 NTU		+0.05nm
28 day	99.9927 ± 0.02				+0.05nm
2 month	99.9923 ± 0.08				+0.07nm
3 month	99.9922 ± 0.034				+0.12nm
6 month	99.99190 ± 0.056				+0.12nm
9 month	99.9915 ± 0.08				+0.14nm
1yr	99.9912 ± 0.06				+0.15nm

TABLE 9: STABILITY STUDIES DATA FOR RS3 IN DOUBLE DISTILLED WATER (NANOSUSPENSION)

Period	Drug content	Viscosity	Sedimentation	Redispersibility	Changes in size
	2-8 °C 25 °C (± 2 °C)				
0 day	99.9937 ± 0.003				0 nm
1 day	99.9937 ± 0.02				0 nm
2 day	99.9937 ± 0.022	0.845 cp ±.05 at room		Not required as no	0 nm
3 day	99.9937 ± 0.034	temperature and 1.07		sedimentation seen	0 nm
5 day	99.9937 ± 0.056	cp at refrigeration	No evidence of any		0 nm
7 day	99.9937 ± 0.076	throughout the shelf	change in turbidity		0 nm
14 day	99.9937 ± 0.11	life	hence no		0 nm
21 day	99.9937 ± 0.8		sedimentation.		0 nm
28 day	99.9937 ± 0.2		Turbidity was 25 ±		0 nm
2 month	99.9933 ± 0.05		5 NTU		0 nm
3 month	99.9933 ± 0.09				0 nm
6 month	99.9933 ± 0.03				+0.02nm
9 month	99.9932 ± 0.13				+0a.02nm
1yr	99.9932 ± 0.0713				+0.02nm



GRAPH 1: SHOWING THE EFFECT OF AGEING AND TEMPERATURE ON DRUG CONTENT AND PARTICLE SIZE

TABLE: DATA SHOWING THE EFFECT OF AGEING AND TEMPERATURE ON % DRUG CONTENT AND SIZE OF RS1, RS2 AND RS3 IN DOUBLE DISTILLED WATER AS THE MEDIUM FOR STORAGE (NANOSUSPENSION)

Formulation period	RS1			RS2			RS3		
	Drug content		Changes in size (nm)	Drug content		Changes in size (nm)	Drug content		Changes in size (nm)
Temperature	2-8 ºC	25 ºC (± 2 ºC)		2-8 ºC	25 ºC (± 2 ºC)		2-8 ºC	25 ºC (± 2 ºC)	
0 day	99.9902	99.9902	0	99.993	99.993	0	99.9937	99.9937	0
1st day	99.9902	99.9902	0	99.993	99.993	0	99.9937	99.9937	0
2nd day	99.9902	99.9902	0	99.993	99.993	0	99.9937	99.9937	0
3rd day	99.9902	99.9902	0	99.993	99.993	0	99.9937	99.9937	0
5th day	99.9902	99.9902	+0.2	99.993	99.993	+0.05	99.9937	99.9937	0
7th day	99.9902	99.9901	+0.5	99.993	99.993	+0.05	99.9937	99.9937	0
14th day	99.9895	99.9896	+0.6	99.993	99.993	+0.05	99.9937	99.9937	0
21st day	99.9892	99.9894	+0.6	99.9929	99.993	+0.05	99.9937	99.9937	0
28th day	99.9888	99.989	+0.6	99.9927	99.993	+0.05	99.9937	99.9937	0
2nd month	99.9983	99.9985	+0.8	99.9923	99.993	+0.07	99.9933	99.9935	0
3rd month	99.998	99.9983	+0.9	99.9922	99.9928	+0.12	99.9933	99.9935	0
6th month	99.9978	99.998	+1	99.9919	99.9926	+0.12	99.9933	99.9935	+0.02
9th month	99.9976	99.9979	+1.6	99.9915	99.9923	+0.14	99.9932	99.9935	+0.02
1 yr	99.9975	99.9977	+2.3	99.9912	99.992	+0.15	99.9932	99.9935	+0.02

***In-vitro* Release Study:** The *in-vitro* drug release profile of all the formulation prepared with Eudragit RS100 is shown in **Table 7**. The release is continuous from the first hour up to 58 h in Eudragit RS100 nanoparticles. The release profile suggests that the pattern obtained is due to the combination of dissolution, diffusion, and erosion of the polymers used in both kinds of the nanoparticles. The drug release rate is slower in later hours in case of Eudragit RS100. But it is evident from the graph that the percentage or the ratio is not much affecting the release rate in any of the formulations. The log concentration versus log time graph and log concentration versus time graph was also plotted to study the nature or profile of the release.

TABLE 11: THE % DRUG RELEASE DATA FOR EUDRAGIT RS100 NANOPARTICLES

Time	Amount of the drug released (in mcg) for RS1	% drug release = (drug release /amount of entrapped) × 100	Amount of the drug released (in mcg) for RS2	% drug release = (drug release /amount of entrapped) × 100	Amount of the drug released (in mcg) for RS3	% drug release = (drug release /amount of entrapped) × 100
1 h	1.24	0.012	3.72	0.0372	98.73	0.98
2 h	13.17	0.131	5.95	0.0595	287.68	2.87
5 h	536.8	5.368	215.8	2.15	1555.5	15.55
10 h	1098.62	10.96	926.0	9.26	3965.11	39.65
15 h	2769.85	27.70	3457.55	34.58	5032.20	50.32
22 h	3958.8	39.59	6683.36	66.83	6909.28	69.09
26 h	5987.37	59.87	7072.37	70.72	7972.37	79.72
28 h	6419.8	64.19	7420.21	74.20	8387.8	83.88
36 h	7239.2	72.39	8135.86	81.36	9007.39	90.07
46 h	8106.99	81.07	8806.99	88.07	9451.42	94.51
50 h	8477.4	84.77	8872.54	88.73	9682.61	96.83
52 h	8724.3	87.24	8976.7	89.47	9727.39	97.27
54 h	9109.3	91.09	9359.26	93.59	9890.67	98.91
55 h	9732.12	97.32	9653.88	96.54	9918.93	99.19
56 h	9744.19	97.44	9703.63	97.04	9929.13	99.29
57 h	9750.96	97.50	9827.21	98.27	9934.54	99.35
58 h	9753.08	97.53	9973.89	99.74	9962.34	99.62

Release Kinetics: The kinetics studies revealed that the release profile was fastest from RS3 formulation. T_{50} was found to be 15 h and 99% of drug released in 55 h. The drug showed sustained release up to 58 h of study. The formulation RS2 showed intermediate release profile the T_{50} was somewhere around 18-19 h and 99% of the drug released in 58 h RS1 formulation showed slower drug release profile with T_{50} been 24-25 h and only 97.53% of drug released in 58 h.

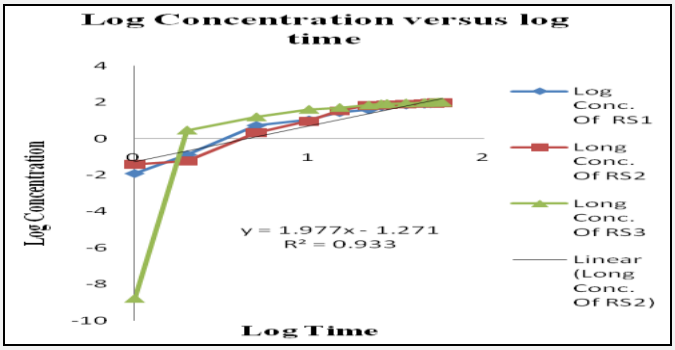
Hence, the concentration of polymer affects the release rate of the drug; however, the drug release pattern was similar. The results reveal that the release was faster in the initial period, *i.e.* 1 to 5 h through the release gradually decreased. Kinetic analysis reveals that the overall release was the second order type. The formulation showed a lag phase of around 1 h. Hence, the prepared Cisplatin nanoparticles are suitable for long term effect, in terms of drug release profile.

TABLE 12: % CONCENTRATION RELEASED DATA WITH THE CORRESPONDING GRAPH

Time	Amount of the drug released (in mcg) for RS1	% drug release = (drug release /amount of entrapped) × 100	Amount of the drug released (in mcg) for RS2	% drug release = (drug release /amount of entrapped) × 100	Amount of the drug released (in mcg) for RS3	% drug release = (drug release /amount of entrapped) × 100
1 h	1.062 ± 0.1	0.012	2.93 ± 0.72	0.0372	82.73 ± 0.38	0.98
2 h	11.59 ± 0.04	0.131	4.70 ± 0.09	0.0595	242.28 ± 0.9	2.87
5 h	474.36 ± 1.17	5.368	169.85 ± 0.2	2.15	1312.73 ± 0.59	15.55
10 h	969.96 ± 0.88	10.96	731.54 ± 0.54	9.26	3347.25 ± 0.76	39.65
15 h	2451.45 ± 0.08	27.7	2919.24 ± 0.3	34.58	4248.01 ± 0.3	50.32
22 h	3503.71 ± 0.51	39.59	5279.57 ± 0.21	66.83	5900.95 ± 0.36	69.09
26 h	5298.49 ± 0.98	59.87	5586.88 ± 0.9	70.72	6729.96 ± 0.09	79.72
28 h	5680.81 ± 1.2	64.19	5861.8 ± 0.75	74.2	7081.14 ± 0.46	83.88
36 h	6406.51 ± 0.22	72.39	6427.44 ± 0.33	81.36	7603.7 ± 0.65	90.07
46 h	7174.69 ± 0.75	81.07	6957.53 ± 0.7	88.07	7978.53 ± 0.18	94.51
50 h	7502.14 ± 0.08	84.77	7009.67 ± 0.42	88.73	8174.38 ± 0.4	96.83
52 h	7720.74 ± 0.66	87.24	7068.13 ± 0.19	89.47	8211.53 ± 0.49	97.27
54 h	8061.46 ± 0.87	91.09	7393.61 ± 0.06	93.59	8349.98 ± 0.77	98.91
55 h	8612.82 ± 0.23	97.32	7626.66 ± 0.01	96.54	8373.61 ± 0.98	99.19
56 h	8623.44 ± 0.56	97.44	7666.16 ± 0.26	97.04	8382.06 ± 0.18	99.29
57 h	8628.75 ± 0.6	97.5	7763.33 ± 0.9	98.27	8387.12 ± 0.54	99.35
58 h	8631.4 ± 0.9	97.53	7879.46 ± 0.04	99.74	8409.92 ± 0.12	99.62

TABLE 13: LOG VALUES TO DETERMINE ORDER OF REACTION

Log time	Log conc. of RS1	Long conc. of RS2	Long conc. of RS3
0	-1.92	-1.42	-8.77
0.301	-.8827	-1.23	0.46
0.699	0.730	0.33	1.19
1	1.039	0.96	1.6
1.176	1.442	1.53	1.7
1.342	1.597	1.82	1.84
1.414	1.777	1.84	1.9
1.447	1.807	1.87	1.92
1.556	1.859	1.91	1.95
1.662	1.908	1.94	1.974
1.698	1.928	1.949	1.979
1.716	1.940	1.954	1.982
1.732	1.959	1.973	1.987
1.74	1.988	1.983	1.995
1.748	1.988	1.988	1.996
1.756	1.989	1.99	1.997
1.763	1.989	1.99	1.998



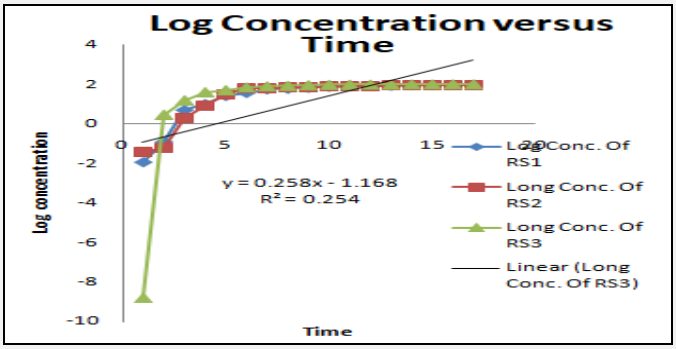
***In-vivo* Evaluation:** SRB Assay does it. The interpretation is as follows: As the concentration increase percent growth decreased in the MA group, and it increases in the FA group.

Formulation FA: As concentration increased percent growth also increased, no LC_{50} GI_{50} and TGI could be established and calculated whereas same drug marketed formulation shows a decrease in growth, as the concentration of drug increased. Less than 10 mg/ml concentration of drug could inhibit 50% of cell growth, TGI concentration causing total inhibition could not found. LC_{50} could not be established, meaning that the drug could

inhibit growth but could not kill cells. Test samples, *i.e.,* FA and MA, has less activity as compared to adriamycin which acted as standard drug control for these cell lines.

TABLE 14: LOG CONCENTRATION VERSUS TIME GRAPH TO DETERMINE ORDER OF REACTION

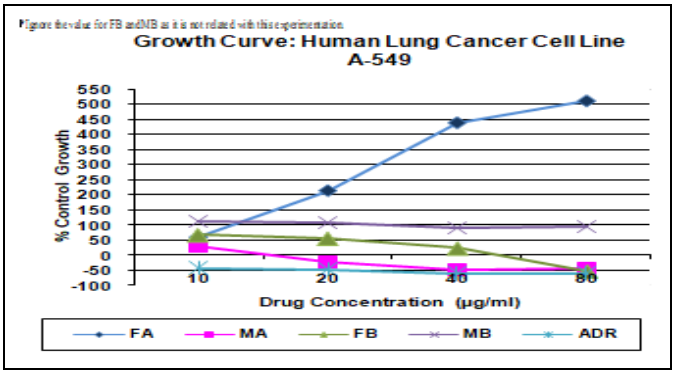
Log time	Log conc. of RS1	Long conc. of RS2	Long conc. of RS3
1 h	-1.92	-1.42	-8.77
2 h	-.8827	-1.23	0.46
5 h	0.730	0.33	1.19
10 h	1.039	0.96	1.6
15 h	1.442	1.53	1.7
22 h	1.597	1.82	1.84
26 h	1.777	1.84	1.9
28 h	1.807	1.87	1.92
36 h	1.859	1.91	1.95
46 h	1.908	1.94	1.974
50 h	1.928	1.949	1.979
52 h	1.940	1.954	1.982
54 h	1.959	1.973	1.987
55 h	1.988	1.983	1.995
56 h	1.988	1.988	1.996
57 h	1.989	1.99	1.997
58 h	1.989	1.99	1.998



FA *vs.* MA: As concentration increased, percent growth also increased; hence, FA had no anti-cancer activity when studied on human lung cancer cell lines and ovarian cancer cell lines. LC_{50} GI_{50} and TGI could not be established whereas same drugs marketed formulation showed a decrease in cell growth as the concentration of drug increased LC_{50} was found to be 72.7 microgram/mili gram, showing that 72.7 microgram/mili gram of drug concentration could kill 50% of drug cancer cells. TGI, *i.e.,* concentration causing total inhibition of cell growth was found to be 13.7 microgram/ml in lung cancer cell lines while no amount could inhibit cell growth in ovarian cancer cell lines. GI_{50} was found to be less than 10 microgram/ml with both cancer cell line, *i.e.* concentration less than 10 micrograms/ml of marketed preparation could cause 50% inhibition of cell growth hence marketed preparations could cause 50% inhibition of cell growth hence marketed preparation of MA was a bit better than nanoparticles of cisplatin formulated.

TABLE 15: SHOWING THE RESULTS OF SRB ASSAY ON HUMAN LUNG CANCER CELL LINE A-549

	Human Lung Cancer Cell Line A-549															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
FA	61.2	244.4	461.6	511.9	54.8	208.6	407.3	442.4	60.5	186.3	447.7	581.1	58.8	213.1	438.9	511.8
MA	31.3	-14.5	-43.5	-40.1	24.8	-20.5	-48.2	-47.7	32.7	-24.7	-49.0	-43.0	29.6	-19.9	-46.9	-43.6
ADR	-32.8	-37.6	-55.6	-52.6	-51.2	-53.2	-60.7	-64.2	-42.7	-47.0	-61.6	-62.6	-42.2	-46.0	-59.3	-59.8

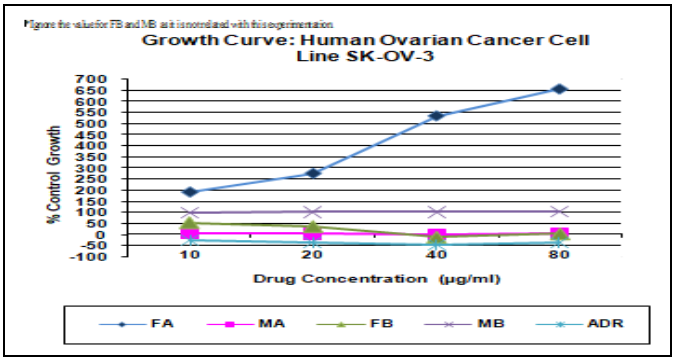


	Drug concentrations (µg/ml) calculated from graph		
A-549	LC_{50}	TGI	GI_{50}*
FA	NE	NE	NE
MA	72.7	13.7	<10
ADR	30.3	<10	<10

GRAPH 2: SHOWING THE RESULTS OF SRB ASSAY ON LUNG CANCER CELL LINES

TABLE 16: SHOWING THE RESULTS OF SRB ASSAY ON HUMAN OVARIAN CANCER CELL LINE SK-OV-3

	Human Ovarian Cancer Cell Line SK-OV-3															
	% Control Growth															
	Drug Concentrations (µg/ml)															
	Experiment 1				Experiment 2				Experiment 3				Average Values			
	10	20	40	80	10	20	40	80	10	20	40	80	10	20	40	80
FA	166.2	310.4	641.7	714.3	188.7	268.5	453.9	636.1	222.6	252.6	514.1	624.5	192.5	277.2	536.6	658.3
MA	10.2	4.5	-1.5	3.3	5.0	-1.5	-5.3	-0.7	-6.8	0.5	3.4	6.1	2.8	1.2	-1.1	2.9
ADR	-25.5	-33.2	-42.6	-39.2	-38.1	-46.6	-61.2	-48.4	-22.3	-31.9	-45.6	-32.9	-28.6	-37.2	-49.8	-40.2



	Drug concentrations (µg/ml) calculated from graph		
SK-OV-3	LC_{50}	TGI	GI_{50}*
FA	NE	NE	NE
MA	NE	NE	<10
ADR	NE	58.3	<10

GRAPH 3: SHOWING THE RESULTS OF SRB ASSAY ON HUMAN OVARIAN CANCER CELL LINE SK-OV-3

Toxicity Studies: Rats administered (i.p.) with eudragit cisplatin nanoparticles shows no mortality in 24 h, the first death observed was after 24 h and the second, third death was after 45 h these animals show the signs like tremors, decreased motor activity, increase in food & water intake, slight loose stool, body rashes, high rise of rat tail is also observed in two rats after 8 h of drug administration. Rats become lazy and dizzy later (15-18 h) after administration. In case of marketed, 4 animals died in 48 h out of which no death seen in first 24 h, three animals died between 24-40 h, and the last animal died after 45 h of administration. Maximum animals seem inactive after administration of the marketed formulation, unlike formulated cisplatin. Animal shows signs like incomplete closing of the lid, straubs reaction, decrease motor activity, slight heavy respiration, increase in water uptake, rashes along the mouth, increased lacrimation, and vomiting. Two animals died after 48 h of administration. 1 animal starts walking lamely after the first hour of administering of dose recovers in a week.

Acute Toxicity Studies: acute toxicity studies were performed in albino rats. The rats were divided into 2 groups: Group 1 received marketed cisplatin injection & Group 2 received cisplatin-containing nanoparticles.

There was no mortality or any other adverse reaction like increase or decrease motor activity, tremors Straub reaction, spasticity, loss of righting reflex, sedation, hypnosis, lacrimation, salivation, depression or stimulation of respiration up to 48 h in both groups.

In Group 1: 5 animals died in 48 h, 3 animals died between 24-40 h after administration of cisplatin injection. The animals in group 1 were inactive showed signs like incomplete closing of the lid, strobes reaction, decreased motor activity, heavy respiration, increase water intake, rashes along the mouth, increase lacrimation and vomiting, one animal started to walk lamely after one hour of administration of cisplatin marketed injection

In Group 2: IP injection of cisplatin nanoparticles: no mortality observed up to 24 h. After that, mortality occurs. Three rats out of six died in 24-45 h of administration of the formulation. The animal showed signs like tremors decreased motor activity, increased food, and water intake, slight loose stool, and body rashes. The high rise of the tale also observed in two rats; hence, formulation seems to be less toxic than marketed injection. As the mortality observed in group 2 is less than group 1.

Safety Studies: some more toxic studies were done to assess the safety of the formulated nanoparticles. The animals were divided into 2 groups I & II, n= 6 each. Group, I received cisplatin injection while group II received formulation containing cisplatin nanoparticles. The animals when observed up to 28

days, the effect on body weight, water intake, food intake, biochemical parameters like SGOT, SGPT, Blood urea, creatinine and hematological parameters that is RBC and WBC count, platelet count, hemoglobin, DLC and hematocrit were studied at different time intervals.

The results are as follows- Interpretation of SGPT & SGOT values and creatinine & blood urea values.

Animals when administered with marketed cisplatin injection showed normal SGOT values up to 28 days however as the time increased the SGOT values decreased up to 10 days, thereafter it increases, and when you become two almost initial value after 28 days while in group 2 animals were administered with cisplatin nanoparticles SGOT values remain normal up to 28 days the value decreased up to 7 days thereafter it increases, and the value becomes almost near to initial value in 28 days. The SGPT parameter, when assist showed that SGPT values increased from the initial value, *i.e.*, 26.73 ± 2.2 to 53.7 ± 1.4 in 28 days. The values remain normal only up to 1 day after that it increased and remain elevated up to 28 days while in cisplatin nanoparticle grow decreased up to 4 days and then it gradually decreased till 28 days. The value remains elevated up to 28 days. The values were lesser then the marketed formulation group, hence cisplatin had an adverse asset on SGPT while cisplatin nanoparticle had the mild protected effect on SGPT values as compared to marketed values

The creatinine values increased in the marketed formulation group, increase up to day 4, and after that, it decreased and came to the nearly normal value in 28 days. While cisplatin nanoparticle formulation group, the values are normal throughout the study, *i.e.*, up to 28 days. In cisplatin marketed formulation group, the marketed blood urea increased up to day 7 and decreased gradually came to normal values within 28 days.

In cisplatin nanoparticle formulation administered group, the blood urea increased up to 10 days and then decreased gradually and came nearly normal in 28 days. Hence, formulated nanoparticle had lesser toxicity as compared to the marketed formulation in terms of kidney function test (KFT) as evident from the following value in the table.

TABLE 17: SHOWING THE CHANGES IN PARAMETERS LIKE WEIGHT, WATER AND FOOD INTAKE IN ANIMALS OF GROUP I ADMINISTERED WITH THE FORMULATED NANOPARTICLES OF EUDRAGIT RS100 AND CISPLATIN

Animal identity		**1**	**2**	**3**	**4**	**5**	**6**
Formulation is given		**Formulated nanoparticles of eudragit RS100 and cisplatin**	**Formulated nanoparticles of eudragit RS100 and cisplatin**	**Formulated nanoparticles of eudragit RS100 and cisplatin**	**Formulated nanoparticles of eudragit RS100 and cisplatin**	**Formulated nanoparticles of eudragit RS100 and cisplatin**	**Formulated nanoparticles of eudragit RS100 and cisplatin**
Original weight in grams	Day 0	250	210	170	150	120	140
	Day 1	240	200	150	135	125	130
	Day 5	225	195	155	130	130	118
	Day 8	270	184	165	122	135	130
	Day 11	274	200	170	136	138	135
	Day 15	280	215	178	145	140	138
	Day 22	285	225	185	145	140	141
	Day 28	285	228	185	145	142	144
Food intake per day in mg	Day 0	48	43.76	41.12	38.34	23.23	37.81
	Day 1	49.32	45.56	45.93	43.3	25.3	38.32
	Day 5	47.73	50.23	49.89	54.4	27.9	36.65
	Day 8	32.22	41.87	44.9	56.43	30	33.33
	Day 11	54.43	44.34	38.39	57	28.3	32.7
	Day 15	57.33	49.33	35.35	58.3	26.7	37.8
	Day 22	65.67	52.66	32.2	58.21	27.19	42.1
	Day 28	60	54.73	39.8	51.2	29.8	45.5
Water intake per day in ml	Day 0	123.56	127.54	143.42	135.22	128.99	132.2
	Day 1	154.44	161.3	148.44	139.7	135.64	141.11
	Day 5	157.3	159.6	150.5	148.33	138.4	144.5
	Day 8	158.45±3.9	155.21	145.8	154.8	141.4	148.9
	Day 11	155.8	152.75	138.9	157.5	143.66	154.6
	Day 15	148.9	157	136.2	153.6	133.9	155.96
	Day 22	141	148.4	132.8	151.3	131.4	150
	Day 28	135.12	136.34	126.67	156.2	133	151.5

TABLE 18: SHOWING THE CHANGES IN PARAMETERS LIKE WEIGHT, WATER AND FOOD INTAKE IN ANIMALS OF GROUP II ADMINISTERED WITH THE MARKETED CISPLATIN

Animal identity		A	B	C	D	E	F
Formulation is given		Marketed Cisplatin	Marketed Cisplatin	Marketed Cisplatin	Marketed Cisplatin	Marketed Cisplatin	Marketed Cisplatin
Original weight in grams	Day 0	230	220	180	180	170	150
	Day 1	220	200	160	170	165	160
	Day 5	210	185	155	150	150	165
	Day 8	190	170	140	145	125	150
	Day 11	185	165	155	150	130	155
	Day 15	210	200	160	155	130	160
	Day 22	225	210	165	160	140	165
	Day 28	225	218	170	170	150	170
Food intake per day in mg	Day 0	40	45	37	40	50.17	35
	Day 1	41	47.3	40.22	37.4	28.7	33.8
	Day 5	45.88	53.33	43.2	39.72	29.07	35.63
	Day 8	45.02	42.86	48.76	45.77	32.03	53.27
	Day 11	46.28	29.45	30.8	53.62	38.22	27.68
	Day 15	40.6	64.44	45.29	34.9	50.34	29.96
	Day 22	39.88	39.88	35.32	41	39.29	62.41
	Day 28	45.97	50.3	38.63	66.1	51.32	35.99
Water intake per day in ml	Day 0	113.26	117.4	134.29	129.9	139.33	142.63
	Day 1	124.7	118.7	158.96	127.3	153.4	139.53
	Day 5	150.87	149.98	161.35	133.26	150.4	151.72
	Day 8	152.22	159.9	142.6	153	158.21	139.4
	Day 11	145.43	147.25	158.3	116.95	136.42	145.8
	Day 15	149.1	148.5	160.22	136.87	143.21	147.66
	Day 22	138.5	145.5	136.3	148.8	128.9	156.49
	Day 28	131.62	132.48	117.88	159.7	129.48	155.02

TABLE 19: SHOWING THE CHANGES OF LIVER FUNCTION OF ALBINO RATS AFTER THE ADMINISTRATION OF EUDRAGIT RS100 AND CISPLATIN NANOPARTICLES (GROUP I) AND MARKETED FORMULATION (GROUP II)

Group Identity	Group I		Group II	
Test	SGOT	SGPT	SGOT	SGPT
Initial values	62.73 ± 3.4	23.7 ± 18.4	67.43 ± 5.4	26.73 ± 2.2
After day 1	52.38 ± 13.5	17.71 ± 1.2	55.9 ± 6.1	25.38 ± 1.9
After day 4	40.0 ± 6.4	48.0 ± 6.0	48.48 ± 8.9	40.0 ± 0.5
After day 7	32.0 ± 3.0	36.0 ± 3.3	44.7 ± 9.4	32.0 ± 1.2
After day 10	43.6 ± 9.0	32.0 ± 1.7	43.33 ± 1.9	43.6 ± 2.3
After day 15	47.6 ± 4.0	35.22 ± 8.9	47.21 ± 3.7	47.6 ± 1.2
After day 22	50.9 ± 12.3	28.08 ± 2.1	58.87 ± 10.7	50.9 ± 1.8
After day 28	53.7 ± 17.9	21.22 ± 12.8	65.59 ± 18.3	53.7 ± 1.4

TABLE 20: SHOWING THE CHANGES OF KIDNEY FUNCTION OF ALBINO RATS AFTER THE ADMINISTRATION OF EUDRAGIT RS100 AND CISPLATIN NANOPARTICLES (GROUP I) AND MARKETED FORMULATION (GROUP II)

Group Identity	Group I		Group II	
Test	Creatinine (mg %)	Blood urea (mg/dl)	Creatinine (mg %)	Blood urea (mg/dl)
Initial values	0.38 ± 0.09	15.16 ± 1.2	0.3 ± 0.1	14.38 ± 8.8
After day 1	0.53 ± 0.09	15.67 ± 2.7	0.63 ± 0.089	26.8 ± 6.2
After day 4	0.64 ± 0.1	23.7 ± 2.9	1.4 ± 0.1	36.81 ± 7.9
After day 7	0.61 ± 0.1	39.0 ± 11.5	1.23 ± 0.1	43.32 ± 1.8
After day 10	0.58 ± 0.09	39.37 ± 11.2	1.27 ± 0.0	36.29 ± 2.0
After day 15	0.55 ± 0.13	36.2 ± 6.0	1.17 ± 0.1	34.14 ± 10.3
After day 22	0.54 ± 0.1	25.0 ± 3.8	1.12 ± 0.1	29.25 ± 2.9
After day 28	0.43 ± 0.06	21.9 ± 2.5	0.86 ± 0.1	20.0 ± 4.3

TABLE 21: SHOWING THE HAEMATOLOGICAL PARAMETERS OF ALBINO RATS AFTER THE ADMINISTRATION OF EUDRAGIT RS100 AND CISPLATIN NANOPARTICLES (GROUP I)

	Initial	After day 1	After day 4	After day 7	After day 10	After day 15	After day 22	After day 28
Hemoglobin (gm/dl)	11.53	10.7	7.2	9.4	9.8	10.3	11.65	12.1
RBC (millions/cmm)	7.0	5.4	3.50	3.69	3.67	4.27	5.55	7.54
WBC /cumm	6800	7900	9899	13900	12600	9800	7700	6700
Platelet (Lakh/cmm)	3.9	3.13	2.3	1.42	1.77	2.0	2.76	3.82
Neutrophils/Polymorphs %)	45	36	30	28	32	35	37	41
Lymphocytes (%)	60	55	62	66	61	58	59	61
Esinophils (%)	02	04	04	04	04	02	02	02
Monocytes (%)	02	02	00	00	00	00	02	02
Basophils (%)	00	00	00	00	00	00	00	00
Haematocrit (%)	37.6	39.0	44.5	48.3	42	39.4	38.8	36.7

TABLE 22: SHOWING THE HAEMATOLOGICAL PARAMETERS OF ALBINO RATS AFTER THE ADMINISTRATION OF CISPLATIN MARKETED (GROUP II)

	Initial	After day 1	After day 4	After day 7	After day 10	After day 15	After day 22	After day 28
Hemoglobin (gm/dl)	11.4	11.7	11.2	11.2	11.6	11.8	12.3	13.6
RBC (millions/cmm)	7.3	6.7	5.95	3.65	4.9	5.2	7.0	8.47
Wbc /cumm	6600	7500	9700	9100	8287	7566	6400	4000
Platelet (Lakh/cmm)	3.3	3.4	3.65	3.80	3.04	2.88	2.0	1.25
Neutrophils/Polymorphs %)	47	34	34	34	45	51	59	63
Lymphocytes (%)	59	60	60	60	55	49	41	35
Esinophils (%)	01	04	04	04	03	02	02	02
Monocytes (%)	03	02	00	02	01	00	00	00
Basophils (%)	00	00	00	00	00	00	00	00
Haematocrit (%)	37.0	38.5	43.5	43.7	44.7	45.9	46.2	46.8

CONCLUSION: The nanoparticles were prepared using a new generation polymer, namely EUDRAGIT. The method was self- modified for the preparation of these nanoparticles. Process optimization and validation were done before the final nanoparticles were obtained. The nanoparticles obtained were smooth and almost round as elicited by SEM and stable more than a year as concluded from stabilities studies. The Nanoparticles which was obtained when undergone *in-vivo* evaluation for the cure and treatment of cancer by Using SRB and MTT Assay. Its Toxicity studies revealed that the formulations were less toxic then marketed formulation.

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