Synthesis and Characterization of Lipid-Polymer Hybrid Nanoparticles for Combinatorial Drug Delivery

By

Kathy Stavropoulos

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Chairperson Christian Schöneich

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John Stobaugh

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Santosh Aryal

Date Defended: June 29, 2011

The Thesis Committee for Kathy Stavropoulos

certifies that this is the approved version of the following thesis:

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Chairperson Christian Schöneich

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Abstract

Overcoming obstacles like multidrug resistance, short circulation half-life, and nonspecific systemic distribution is an ongoing challenge in cancer therapy. One application to address these concerns is to engineer a drug delivery vehicle that has versatile functionality, good serum stability, circulates in the body long enough to reach the targeting tissues, and is biocompatible. A promising formulation platform that embodies these features is the lipid-polymer hybrid nanoparticles. The surface characteristics of these nanoparticles such as charge, lipid density, and targeting ligands can be modified to allow for specific cellular uptake, controlled drug releases kinetics, and enhanced pharmacokinetics. In this work, it was found that the hybrid nanoparticles could easily be fabricated with negatively and positively charged lipids in order to change the overall surface charge. The particle size remained in the desirable range and the distribution was narrow. The lipid-polymer hybrid nanoparticle by design has the capacity to co-encapsulate hydrophobic and lipophilic drugs. To investigate, camptothecin and a cisplatin derivative were dually loaded within the hybrid nanoparticle system. This combination formulation was characterized by dynamic light scattering for particle size, zeta potential, and polydispersity index as well as in vitro drug release and cytotoxicity. The particle size was below 100 nm and the distribution was narrow. The release studies showed that the addition of the two drugs within the lipid-polymer hybrid nanoparticle system did not affect the release profiles of the individual drugs. The ability for co-encapsulation and the similar overall drug release profiles for camptothecin and cisplatin derivative in the combination compared to single drug loaded controls valuates this already useful drug delivery platform.

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Chapter 1: Literature Review

1.1 Review of Nanomedicine

In recent years, the application of nanotechnology has been translated to medicine. Nanotechnology encompasses the design, synthesis, and characterization of materials and or devices, which are functionally organized in at least one dimension on the nanoscale [1]. The use of these nanoscale or nanostructured materials in medicine, termed as nanomedicine, has unique medical properties and effects owing to the small size (1 – 1000 nm) and structure [2]. The ability to engineer and control materials in this size rage results in new medical efforts, innovative chemistry techniques, and novel manufacturing approaches [2]. Nanomedicine has the capacity to change the landscape of healthcare and drug delivery by enhancing the developability of biologically active drug candidates with poor pharmaceutical properties such as solubility and circulation half-life [3]. Nanomedinces and nanomaterials are engineered to have specific functions, which utilize the physical properties and characteristics for diagnosis and treatment of disease [4]. These materials are able to be used as carriers to cross membranes, mediate molecular interactions, and detect molecular changes [4]. Nanomaterials have a high surface to volume ratio. This increased surface area can be coated or tagged with other molecules, which results in the formation of multifunctional nanomaterials [4]. Nanomaterials can be engineered to have different shapes, sizes, surface chemistry, particle density, and chemical compositions [4]. Because of their design, nanomedicines have applications in drug delivery, in vivo and in vitro diagnostics, biomaterials, active implants, in vivo imaging, biosensing, cell labeling, and tissue engineering [1-3, 5,6].

In vivo imaging employs the use of magnetic nanoparticles, quantum dots, fluorophores, and carbon nanotubes [2,3,5,6]. An example is Gastromark (ferumoxsil®),

which is a marketed product composed of superparamagnetic iron oxide nanoparticles used as a contrast agent for magnetic resonance imaging [2]. Fluorescent quantum dots are nanocrystals that have higher extinction coefficients than traditional fluorophores, which makes this technology useful for imaging [6]. Carbon nanotubes can act as biocompatible supportive substrates that can incorporate fluorophores and other molecules [7]. Using nanomaterials for in vivo imaging is a faster, less invasive, and a more accurate way to diagnose diseases and to monitor disease states and progression [3]. In the future, these types of imaging probes may be able to assist surgeons in locating tumors within the body and to identify adjacent structures [3].

In vitro diagnostics is another application for nanomedicine, which uses nanoparticles, nanowires, nanotubes, nanoarrays, and cantilevers [2,3]. Lateral flow assays are marketed products that utilize colloidal gold to test ovulation, HIV infection, and pregnancy [2]. In this case, an antibody for a specific analyte is conjugated to the nanoparticle surface. Gold nanoparticles are widely used because they have good stability, which avoids the chance of false positive readings [2]. With the use of these materials, disease detection can be quick, high throughput, and more accurate by using biomarkers with higher sensitivity [3]. In the future, novel analytes could be measured such as Alzheimer's plaques [2]. Using nanomaterials for in vitro diagnostics is advantageous because they can improve sensitivity, reduce cost, and consume less of the sample [2,3].

Biomaterials have mechanical properties than can be used as medical implants, dental restoratives, and bone substitutes [3]. One example of a biomaterial is the nanoparticle composite found in the dental restorative Filtek Supreme®, which is a marketed product produced by 3M [2]. Vitoss® is a marketed nano-hydroxyapatite based

product that is used in the repair of bone defects [2]. Another example of biomaterials in the market is Anticoat®, which is a silver nanoparticle based wound dressing [2].

Nanomedicines have been especially successful as drug delivery vehicles. This may be due to the fact that diseases originate at the molecular level, which is on the nanoscale and can be caused by gene mutations, misfolded proteins, viral and bacterial infections, cell misfunction, and cell miscommunications [4]. In order to address these modes of disease, nanocarrier delivery systems were developed. Nanotechnology formulation platforms include liposomes, nanoparticles, polymeric micelles, dendrimers, nanocantilevers, carbon nanotubes, aptamers, quantum dots, and polymer conjugates [8]. Liposomes consist of a phospholipid bilayer and an aqueous core for drug encapsulation of water-soluble molecules. Marketed liposomal products include Doxil and Myocet (liposomal doxorubicin), Ambisome (liposomal Amphotericin B), DaunoXome (liposomal daunorubicin) and Depocyt (liposomal cytarabine) [2]. There are also several examples of marketed products that are polymer conjugates. Polyethylene glycol (PEG) is conjugated to a molecule in order to increase circulation time [9]. Pegasys (PEG-α-interferon-2a) and PEG-Intron (PEG-α-interferon-2b) are both therapies for hepatitis C in which PEG is conjugated to a protein [2]. These marketed products are considered first generation nanosystems because the drug is contained within a system used for passive targeting [3].

Nanomedicine can be a solution for cancer therapy where the current treatments have some problems that include non-specific systemic distribution of the drug, inadequate drug concentration reaching the target site, normal tissue toxicity, and drug resistance [8,10]. Nanomedicines can be used to overcome these obstacles that

conventional medicines cannot address. Because of their size and surface properties, nanomedicines can accumulate in tumor sites due to the enhanced permeability and retention (EPR) effect [4]. Nanomedicines have the capacity to encapsulate multiple drugs in order to yield combinatorial delivery, increase circulation time, and exhibit controlled drug release kinetics [11]. This allows for improved dose scheduling, which leads to patient compliance.

The impact of nanotechnology for drug delivery is that the characteristics of the vehicles such as size, charge, surface hydrophobicity, ligand type, and density of ligands on the surface can enhance pharmacokinetic properties such as circulation half-life and biodistribution while also improving pharmaceutical properties such as drug solubility [12]. Because of this, nanotechnology is beneficial for the pharmaceutical industry since it can provide life-style extensions for drugs after patents have expired, new classes of drug therapeutics can be developed, and the biologically active molecules that have poor pharmaceutical properties can be re-investigated [3,12].

1.2 Nanoparticle Drug Delivery

Within the body there are multiple biological barriers in which drug delivery vehicles need to be engineered to overcome. There are different mechanisms for delivery vehicles to target tissues and be sequestered by specific cells. One particular biological process that is important for nanomedicine delivery is endocytosis (Figure 1.1). For endocytosis to occur, a molecule or material must be recognized in the bloodstream via specialized absorptive proteins [13]. The recognized material undergoes adhesion onto the cellular membrane. After adhesion, the material becomes engulfed into the cell. There

are specific mechanisms of endocytosis and it has been reported in the literature that poly(lactic-co-glycolic acid) (PLGA) nanoparticles enter cells through clathrin-mediated endocytosis as well as caveolae-independent pathways, depending on the cell type [13]. Bare PLGA nanoparticles have negative surface charge and since cell membranes are negatively charged, these nanoparticles would internalize at a slow rate as a result of charge repulsion. Clathrin-mediated endocytosis occurs when a pit in the cell membrane is formed by the polymerization of clathrin-1 and other assembly proteins [13]. Ligands that bind to receptors get engulfed in this pit. An enzyme pinches off the assembled vesicle, and then the clathrin coating is removed. The vesicle fuses with endosomes and gets sorted by the cell [13]. It is also reported that positively charged nanomaterials enter cells via clathrin-mediated endocytosis at a faster rate than negatively charged nanoparticles [13]. Nanoparticle surface charge can be designed according to the desired route of cellular transport.

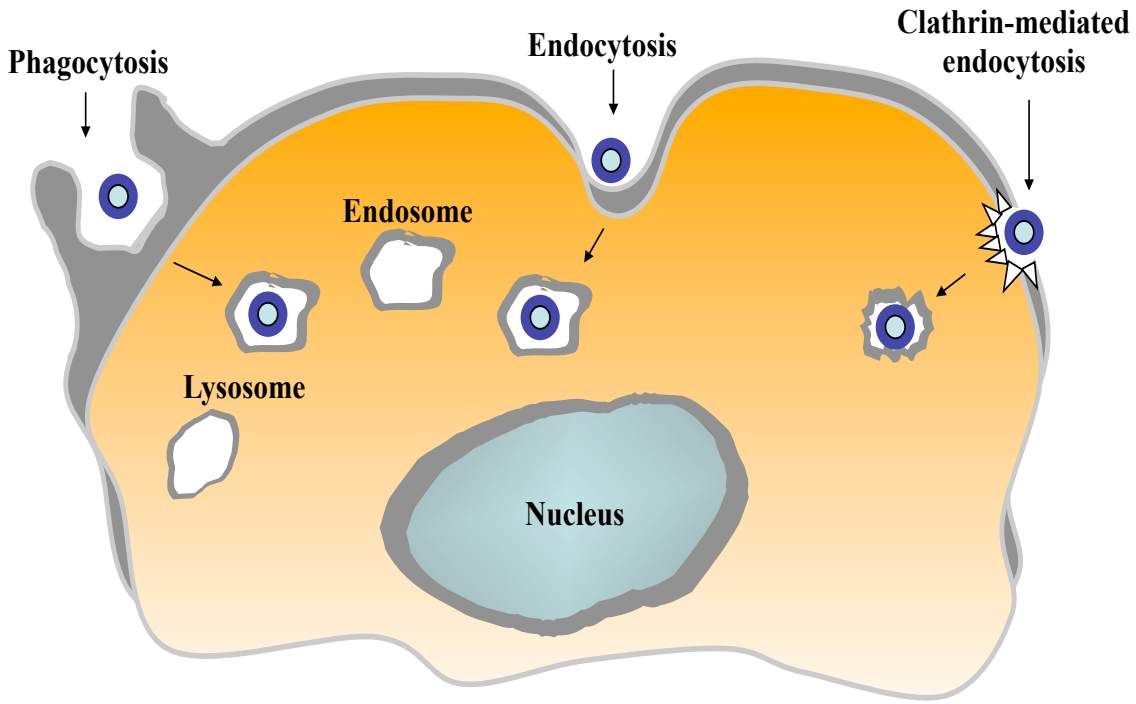

Figure 1.1. This diagram shows a schematic representation of endocytotic processes.

The blood brain barrier (BBB) is another biologically barrier that is challenging for conventional therapeutics to cross in order to treat central nervous system (CNS) diseases. The blood brain barrier is comprised of endothelial cells, pericytes, astrocytes, and a basal membrane [14]. The brain capillaries are covered by pinocytes and microvessel endothelial cells which makes for a compact structure with tight junctions [14]. Molecules are able to cross the BBB by diffusion mechanisms as long as the molecules are lipophilic, not ionizable at physiological pH, and have a size below 400 kD [14]. Due to their small size and design, nanoparticles have the capacity to penetrate through the tight junctions in the BBB and protect the drug from enzymatic degradation [14]. These advantages make the nanoparticle drug delivery platform an attractive option for treatment of CNS diseases including cancer.

In the treatment of cancer, the biological barrier for chemoagents to overcome is permeation through the cancer cell networks. Cancer cells that make up tumors have unique biology and anatomy that differs form healthy cells [11]. A way to take advantage of this unique biology and anatomy is via the enhance permeability and retention (EPR) effect (Figure 1.2). Tumors and cancer cells proliferate quite rapidly and don't have enough oxygen and nutrients to sustain such a quick growth rate, so they grow blood vessels in disorganized heterogeneous networks that lead to a large vascular density [15]. This biology is shown in Figure 1.2. This disorganization provides gaps in the endothelium cell-cell junctions. Tumor blood vessels also have larger pores, which increases the permeability and hydraulic conductivity causing the ERP effect [11]. In addition to the defective vascular arrangement and extensive blood vessel growth, cancer cells also have impaired lymphatic drainage [15]. Nanoparticle drug delivery vehicles can

reach these cancer cells and tumor cells by passive or active targeting and take advantage of the EPR effect. Passive targeting is when the drug and or drug carrier accumulates at the desired site owing to physical and chemical properties of the formulation or pharmacological factors [8]. Formulation factors include particle size distribution while the pharmacological factors are the leaky tumor vasculature, the EPR effect, and the tumor microenvironment, which is acidic due to glycolysis [8]. The other approach is active targeting, which involves attaching a targeting ligand to the nanocarrier. This targeted nanoparticle internalizes within the cancer cell through receptor-mediated endocytosis [8,13].

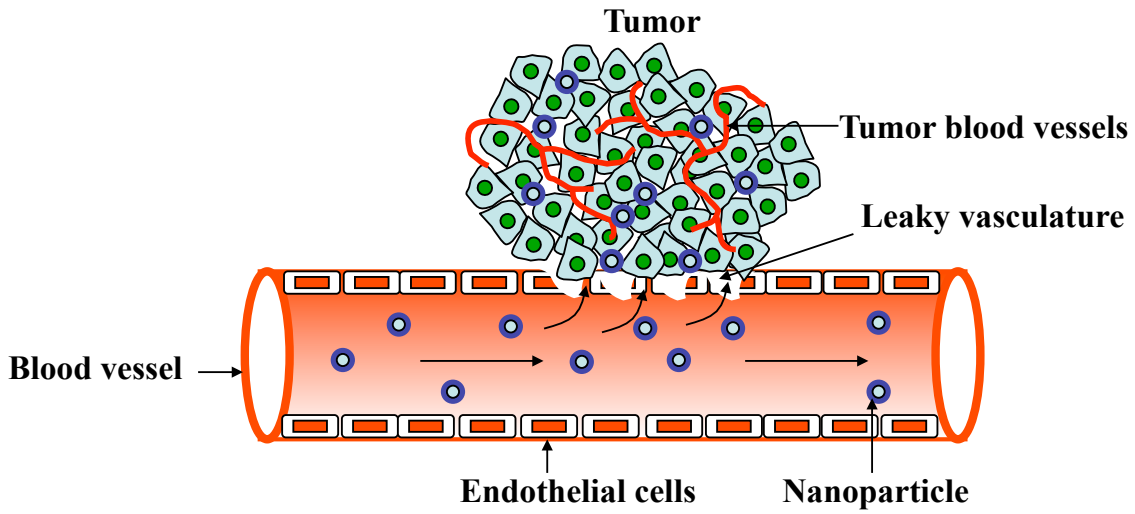

Figure 1.2. Schematic representation displaying the cells and blood vessels involved with the enhanced permeability and retention (EPR) effect.

To date, the most common cancer treatments are chemotherapy, radiation, and surgery. The challenging aspects involved with cancer therapy include nonspecific systemic distribution, low drug levels reaching the tumor site, cytotoxicity, poor stability, and multidrug resistant tumor cells [8,10,16]. Multidrug resistance (MDR) is a major hurdle in cancer therapy because it decreases the efficacy of drugs through multiple mechanisms [17]. This phenomenon involves an active efflux of a large range of cytotoxic drugs out of the cytoplasm by membrane-bound transporters [16]. One example is the P-glycoprotein (P-gp), which is an active membrane-bound efflux pump [18]. Over-expression of P-gp and other membrane transporters can lead to MDR [19]. Other cellular mechanisms that contribute to MDR are drug molecule reactions with intracellular nucleophiles like glutathione, repair of drug-induced damage to the DNA, altered proteins that affect apoptotic pathways, and an altered drug target [16,17]. Non-cellular events that can lead to MDR include high interstitial pressures at the tumor site, which decreases drug permeability, lower pH, hypoxia (drugs generating free radicals), and the extracellular matrix effect [16]. Therapeutic materials can also be removed from the systemic circulation by the mononuclear phagocyte system that comprises of kupffer cells in the liver and macrophages in the spleen and bone marrow [20]. Nanoparticle drug delivery vehicles can be designed to address these challenges associated with current cancer treatment.

Nanosystems are distinct from other cancer therapeutics because the nanocarrier itself can also have therapeutic effects along with the actual drug [8]. Nanoparticles can be designed to carry large payloads, have attached targeting ligands, encapsulate multiple drugs for combinatorial therapy, and have the ability to bypass drug resistance

mechanisms [8]. Material selection is an important consideration for nanomedicines. Biodegradable, biocompatible, and physiological lipids are chosen for formulation development in an attempt to reduce immunogenicity and minimize toxicity [21]. Colloidal drug carriers such as micelles, nanoemulsions, nanosuspensions, polymeric nanoparticles, and liposomes are formulation platforms that are used to address drug solubility and stability issues [21]. One example of a nanoparticle delivery vehicle is a dendrimer, which is a biodegradable branch-like structure that consists of a core (two or more reactive groups) with repeated units covalently bound to the core and peripheral functional groups [22]. Drugs can be encapsulated or conjugated to the dendrimer and delivered to tumor sites through the EPR effect or by using targeting ligands like peptides and antibodies [22]. Another example is quantum dots, which are nanocrystals that have improved fluorescent properties over traditional fluorophores and can be used as drug carriers or as tags for other drug carriers [23]. Liposomes have a lipid bilayer in which the surface characteristics can be modified by lipid type and lipid charge. Cationic liposomes are established in the literature to have antimicrobial action due to the adsorption of the positively charged lipid bilayer onto the bacterial cell membrane, which changes the membrane surface charge from negative to positive and induces apoptotic cell death [24]. With liposomes and polymeric nanoparticles, multiple drugs can be co-encapsulated in a single system for combination delivery.

Combination delivery has been used in malaria, HIV/AIDS, and cancer [25]. This approach is employed in cancer therapy to minimize multidrug resistance and reduce cytotoxicity. Combining chemoagents hits different targets and displays different toxicity profiles, which can improve efficacy or have comparable efficacy and decreased toxicity

[25]. An example of biochemical synergy for the treatment of nonlymphocytic leukemia is the combination of anthracycline daunorubicin (a DNA intercalator) with ara-C (a DNA polymerase inhibitor), which interferes with DNA repair and DNA synthesis [25]. For colorectal cancer, administering leucovorin prior to 5-fluorouracil enhanced that ability to bind and block the action of thymidilate synthetase [25]. From a patient compliance view, it would be elegant to contain multiple drugs within one delivery vehicle. This could lead to a more convenient dose-scheduling regimen and would improve patient quality of life. Having this in mind, nanodelivery platforms have to be simple, scalable, broad-based, and meet Food and Drug Administration (FDA) requirements [26]. Formulation platforms that are successful should be engineered with these specific properties: biocompatibility, biodegradation, encapsulation efficiency, colloidal stability, improved pharmacokinetics, and controlled drug release kinetics [26]. Lipid-polymer hybrid nanoparticles as a drug delivery platform is one that embodies the lipid shell characteristics of a liposome and the hydrophobic core of a polymeric nanoparticle. The fabrication process is reproducible and encapsulation efficiency is sufficient for camptothecin (CPT) and a cisplatin derivative, which both show cytotoxicity in A2780 human ovarian carcinoma cells. Hydrophobic drugs can be encapsulated in the polymeric core while targeting ligands can be tagged to the lipid shell. The robustness and versatility makes this formulation platform practical for cancer treatment and it can be loaded with multiple drug agents for combinatorial delivery.

1.3 Lipid-Polymer Hybrid Nanoparticles

Nanomedicine drug delivery systems for cancer therapy are designed to protect the drug from inactivation due to the biological environment, protect non-pathological tissues from non-specific toxic actions of the drug, and to change or control drug pharmacokinetics [9]. Lipid-polymer hybrid nanoparticles are a nanomedicine formulation platform that can be used for cancer treatment. The anatomy of a hybrid nanoparticle consists of a hydrophobic poly(lactic-co-glycolic acid) (PLGA) polymer core, a lipid monolayer surrounding the core, and a lipid-PEG (for example: 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-carboxy(poly(ethylene glycol)) 2000 (DSPE-PEG-COOH)), which is distributed within the lipid monolayer to form a polyethylene glycol (PEG) corona [27]. The polymeric core affects drug encapsulation and release. Drug release from the nanoparticles begins with diffusion processes, followed by erosion, then swelling of the matrix [28]. The polymer degrades due to hydrolysis and the degradation rate depends on the polymer composition and molecular weight [28]. The lipid shell serves the purpose as a biocompatible shield, a template for surface modifications, and a barrier for preventing water-soluble drugs from leaking out of the core [29]. The corona affects biodistribution and circulation half-life. The PEG corona provides electrostatic and steric stabilization as well as a protective layer from adsorptive recognition proteins in the bloodstream [9, 27]. There are multiple fabrication methods for preparing lipid-hybrid nanoparticles found in the literature which include, but are not limited to solvent – evaporation, emulsion method, nanoprecipitation followed by self – assembly, and a sonication method [30-32].

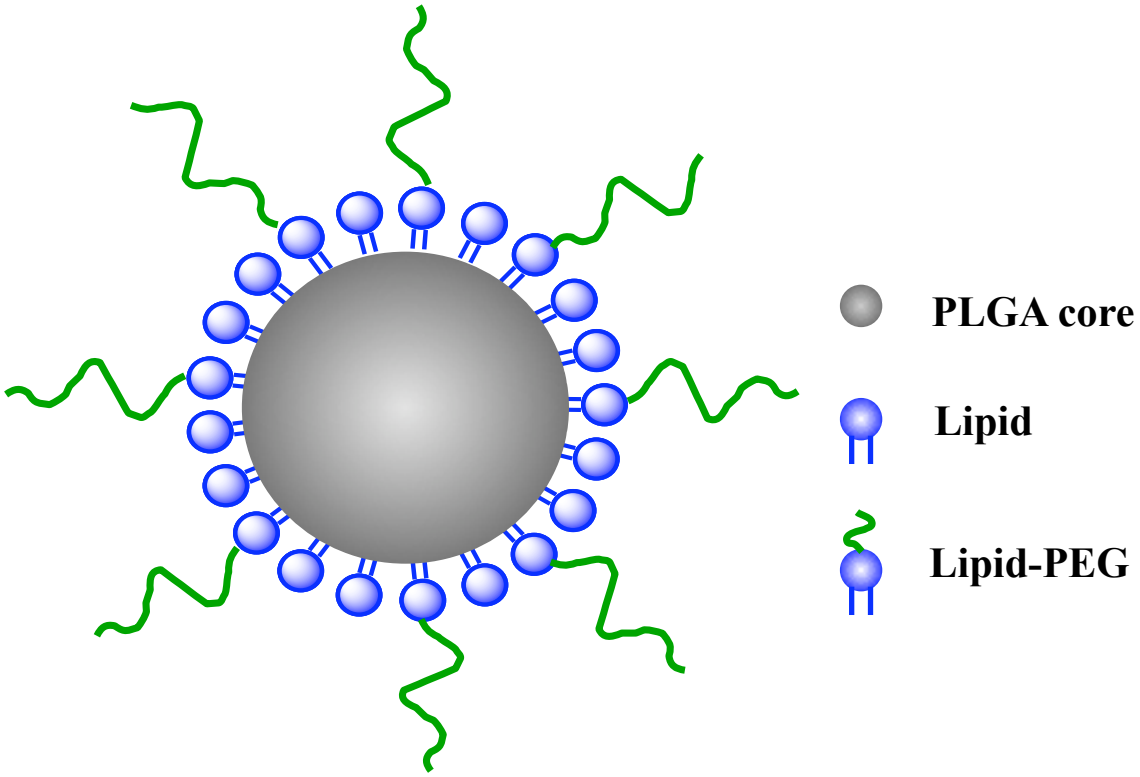

Figure 1.3. This diagram shows a representation of the anatomy of a lipid-polymer hybrid nanoparticle.

The versatility of this lipid-polymer hybrid nanoparticle platform allows for surface chemistry modifications. Li et. al., used cationic lipids in their hybrid nanoparticles in order to form a DNA complex for gene delivery [33]. The end group on the PEG that makes up the corona can be changed from a carboxyl group to an amine or a methoxy group in order to change the surface zeta potential [34]. In a study done by Salvador-Morales et. al., it was shown that the surface chemistry of the hybrid nanoparticles affects human plasma and serum absorption patterns by inducing different levels of compliment activation [34]. They also performed coagulation studies that showed no hybrid nanoparticle formulation with the modified surface groups activated the coagulation cascade [34]. These studies exhibited the potential for the lipid-polymer hybrid nanoparticles to be a viable immunocompatible delivery option. Another type of surface chemistry modification is the addition of targeting ligands, which are used to increase cellular uptake and accumulation in the tumor sites. Different types of ligands are used to target hybrid nanoparticles to cancers cells such as antibodies, antibody fragments, proteins, small molecules, aptamers, and peptides [35]. The ligands should induce receptor-mediated endocytosis and have the correct conformation to maintain affinity for its corresponding receptors [35]. Also the ligand must not disturb the steric and electrostatic stabilization that comes from the PEG corona. If the ligand concentration is too high then it mitigates the stealth action of the protective PEG layer [35]. An example from the literature using targeting ligands comes from Hu et. al., in which they conjugated a half-antibody to lipid-polymer hybrid nanoparticles for use in pancreatic cancer treatment [36]. Another example from the literature is the use of wheat germ agglutinin (WGA) on the surface of PLGA nanoparticles for targeted intracellular

delivery of paclitaxel [37]. WGA binds to the N-actetylglucosamine and the sialic acid residues on the cell membrane, which leads to cellular internalization by receptor-mediated endocytosis [37].

With all the functionality that is available to lipid-polymer hybrid nanoparticles, another opportunity can be used with this system for combinatorial delivery. In this system a hydrophobic drug can be encapsulated in the PLGA core while a lipophilic drug can be incorporated within the lipid shell. Co-formulation of multiple drugs in a single system has the advantage of delivering the correct drug ratio to the target of interest as well as synergistic therapeutic effects, suppressed drug resistance, and a timed drug exposure control [38]. One proof of concept example by Kolishetti et. al., is the encapsulation of docetaxel with a cisplatin prodrug conjugated to the polymer to treat prostate cancer cells [38]. Similarly, Aryal et. al., have demonstrated the combinatorial drug delivery system in which paclitaxel (a hydrophobic drug) and gemcitabine (a hydrophilic drug) were conjugated by a hydrolysable linker, followed by the encapsulation of the drug conjugate into a hybrid nanoparticle [39].

Nanoparticle formulation platforms have several advantages in delivering cancer therapeutics. They provide a system that improves drug solubility, increases half-life circulation due to evasion of the mononuclear phagocytic system, enhances the drug accumulation in target cells, provides a stable drug release, and reduces efflux pump-mediated drug resistance [40]. In this work, the next steps were taken with the nanoparticle platform to optimize surface charge in order to take advantage of cellular uptake mechanisms and to encapsulate multiple drugs within a single system in order to improve the cytotoxicity and drug release kinetics.

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Chapter 2: Synthesis and Characterization of Hybrid Nanoparticles

2.1 Introduction

Nanoparticles have been used in medicine for diagnostic therapy. Two major classes of nanocarriers used for delivering therapeutic drugs in disease therapy are biodegradable polymeric nanoparticles and liposomes [1]. The advantageous size range (10 – 200 nm) inherent to nanoparticles is favorable for endocytotic intercellular uptake [2]. This capability to permeate through cell walls makes the polymeric nanoparticle platform practical as a nanomedicine for cancer therapy [3]. These vehicles can accumulate in the tumor site through leaky tumor vascular structures, which is useful for prolonged drug exposure to the tumor site [4].

Polymeric nanoparticles are practical drug delivery vehicles because they can be used to encapsulate hydrophobic drugs, which would otherwise have too low aqueous solubility for other drug delivery systems [1]. Circulation time in the body can be increased with polyethylene glycol (PEG) as part of the corona of the particle, which enables the particle to avoid phagocytosis mechanisms and reach the targeted tissues to release the drug [5]. Surface modifications can be engineered to the hybrid nanoparticle platform in order to target specific tissues and improve cellular uptake. For instance, the effect of particle charge has on impact of the mode of action of cellular uptake. If the nanoparticles are positively charged, they would enter the cell by means of clathrin-mediated endocytosis [6]. Negatively charged nanoparticles would internalize at a slower rate since the cellular wall itself is negatively charged [6].

In this work, the hybrid nanoparticle platform that was developed by Fang et. al. was used [1]. These hybrid nanoparticles were fabricated using the sonication method in

order to modify surface characteristics of the particles. By doing this, the drug can be targeted for delivery to specific tissues and have improved cytotoxicity as well as an enhanced pharmacokinetic profile, which could lead to more efficacious therapy and patient compliance [7].

2.2 Materials and Methods

Ester-terminated poly(DL-lactic-*co*-glycolic acid) (PLGA) (inherent viscosity = 0.82 dL/g) was obtained from LACTEL Absorbable Polymers (Pelham, AL). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-carboxy(poly(ethylene glycol)) 2000 (DSPE-PEG-COOH), L-α-phosphatidylcholine (Egg Chicken, EGG PC), L-α-phosphatic acid (Egg, Chicken) (EGG PA), and 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt) (DOTAP) were obtained from Avanti Polar Lipids (Alabaster, AL). Acetonitrile and all other solvents were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification.

2.2.1 Hybrid Nanoparticle Synthesis

Hybrid nanoparticles were prepared using the sonication method as described by Fang et. al [1]. The surface zeta potential of these nanoparticles was tuned by varying the nature of the lipids. Prior to the synthesis, the stock solutions of all the materials were prepared as shown in Table 1 and kept under 4 ^{0}C for further use. These stock solutions were used only for the period of three weeks and there after the solutions were replaced by freshly prepared solutions. In a typical hybrid nanoparticle synthesis, 25 μg of EGG-PC and 250 μg of DSPE-PEG dissolved in 275 μL of 4 % EtOH were diluted to 3.3 mL with water. To this solution, 1 mg of PLGA dissolved in 400 μL of acetonitrile (ACN)

was added under sonication. The calculated amount of deionized water was added to adjust the final volume to 4 mL and the volume ratio of aqueous to organic solution was 9:1. The mixture was sonicated in a capped glass vial for 5 min using a Fisher Scientific (FS30D) bath sonicator at a frequency of 42 kHz and power of 100 W. The solutions were washed 3 times with deionized water using a Millipore (Amicon Ultra) centrifuge filter with a molecular weight cutoff of 10 kDa. The samples were concentrated down to 1 mg of PLGA polymer to 1 mL of particle solution. All other formulations were prepared similarly as shown in Table 1 by changing the lipid accordingly to obtain hybrid nanoparticles with various surface zeta-potential. Control lipid vesicle solutions were prepared to confirm the surface charge of each lipid. Briefly, 100 μg of lipid was added to 900 μL of water and was vortexed. The resulting solutions were measured for zeta potential values as described in the following section.

Formulation	Amount lipid (1 mg/mL)	Amount DSPE-PEG-COOH (1 mg/mL)	Amount PLGA (2.5 mg/mL)	Amount Water
1	0 μL	250 μL	400 μL	3350 μL
2	25 μL	250 μL	400 μL	3300 μL
3	50 μL	250 μL	400 μL	3250 μL
4	100 μL	250 μL	400 μL	3150 μL

Table 1. Hybrid nanoparticle formulation compositions.

2.3 Characterization

After successful synthesis of various lipid coated hybrid nanoparticles, the particles were characterized using different state-of-art analytical technique including dynamic light scattering (DLS), scanning electron microscope (SEM), and transmission electron microscopy (TEM).

2.3.1 Particle Size and Polydispersity Index (PDI)

Particle size measurements were performed by using dynamic light scattering (DLS) technique (Malvern Zetasizer, ZEN 3600). Three subruns were carried out per measurement, and the average values were taken.

2.3.2 Zeta Potential

Zeta potential measurements were taken using the Malvern Zetasizer (ZEN 3600) in which the electrophoretic mobility on the surface of the nanostructures was measured. The measurements were carried out at room temperature with the backscatter angle of 173°. Three subruns were carried out per measurement and the average values were taken.

2.3.3 Scanning Electron Microscopic (SEM) Analysis

Scanning electron microscopy is the technique used to look at morphology and surface structure of the materials. Samples for SEM were prepared by dropping 5 μL of a nanoparticle solution onto a polished silicon wafer. After drying the droplet at room temperature overnight, the sample was coated with chromium and then imaged under Phillips XL 30 ESEM.

2.3.4 Transmission Electron Microscopic (TEM) Analysis

Transmission electron microscopy is the technique used to look at the internal structure of the materials. In order to understand the internal structure of hybrid nanoparticles, a drop of the nanoparticle solution at a concentration of 4 μg/mL was deposited onto a glow-discharged carbon-coated grid. Five minutes after the sample was deposited the grid was rinsed with ten drops of distilled water. A drop of 1% uranyl acetate stain was added to the grid. The grid was subsequently dried and visualized using a FEI 200KV Sphera microscope.

2.4 Results and Discussion

Several synthetic polymers approved by the US FDA, such as poly(lactic *co*-glycolic acid) (PLGA) and polycaprolactone (PCL), have been used in biomedical applications including drug delivery systems and tissue engineering [8]. In drug delivery, the hydrophobic and hydrophilic block copolymers that self-assemble into nanostructures have an immediate application. In addition, the nanoparticles can be sealed with biomolecules such as lipids, which can enhance the surface property of these nanoparticles. The lipid-polymer hybrid nanoparticles are capable of having a sustained drug-release profile, and higher loading capacity for poorly water-soluble drugs.

The hybrid nanoparticle platform has the versatility to be engineered for specific needs because its ease of tuning the lipid constructs on the surface. Because of these unique characteristics, hybrid nanoparticles have attracted interest from academia and industry, even though they are still in a relatively early stage of development [8]. In the current formulation, various lipids depending on their cationic, anionic, and neutral

charge have been employed in order to synthesize nanoparticles that show promise as drug delivery vehicles.

As shown in Figures 2.1, 2.2, and 2.3, the hydrodynamic size of these hybrid nanoparticles exhibit an average size of ~100 nm. All the nanoparticles prepared herein are uniform and unimodel in size distribution with a narrow polydispersity index (PDI). The formation of uniform nanoparticles was further characterized using electron microscopy. Both surface and internal structures suggested the formation of well-defined spherical nanoparticles. The SEM image (Figure 2.4) shows that the hybrid nanoparticles have a spherical morphology. The shape of the particle will play a key role in pharmacokinetics, drug release, and cell uptake. It also confirms that there is a narrow particle size distribution within the formulation with particles having ~100 nm size. On the other hand, the TEM micrographs further confirm the formation of lipid coated polymeric nanoparticles. The TEM micrograph (Figure 2.5) showed the spherical units that were sealed with thin lipid monolayer. The negative staining clearly indicates the higher contrast on the circumference of the nanospheres that confirms the presence of lipid monolayer. It is evident from TEM image that during the nanoprecipitation process the hydrophobic PLGA polymer amassed to contribute the core of the nanoparticles whereas lipid are assembled onto the surface of the nanoparticles.

As shown in Figure 2.7, the surface potential of the hybrid nanoparticles that are shielded with cationic lipid i.e., DOTAP shows the decrease in negative zeta-potential whereas that of the anionic lipid EGG PA (Figure 2.8) shows an increase in negative zeta-potential. Although the overall charge of hybrid nanoparticles was negative due to the presence of –COOH group in DSPE-PEG-COOH, the tuning the amount of the

second lipid component tunes the over all charge. As shown in Figure 2.9, EGG PC, which is a neutral lipid didn't contribute significantly to tune the surface zeta-potential due to DSPE-PEG-COOH. This further confirms the capacity to modify the zeta-potential by changing the nature and the concentration of the lipids.

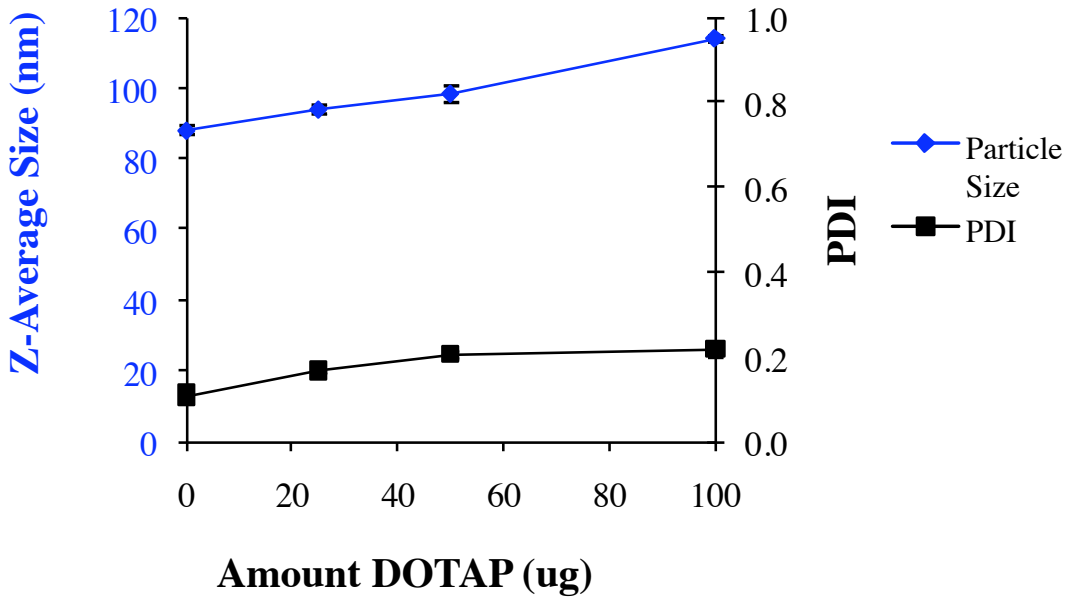

Figure 2.1. Effect of DOTAP concentration on nanoparticle size and polydispersity index (PDI)

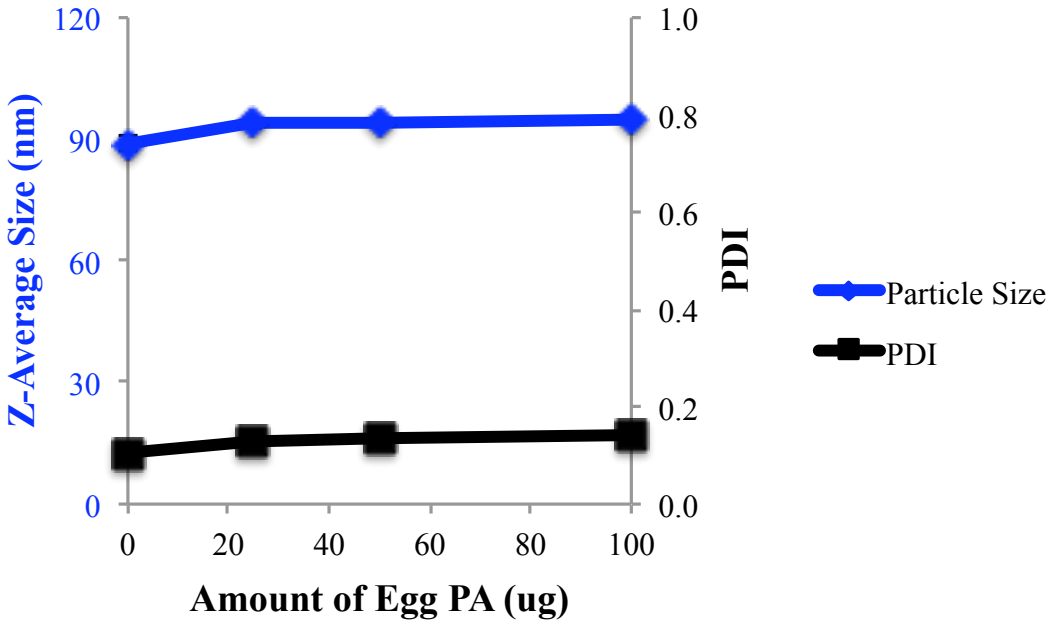

Figure 2.2. Effect of EGG PA concentration on nanoparticle size and polydispersity index (PDI)

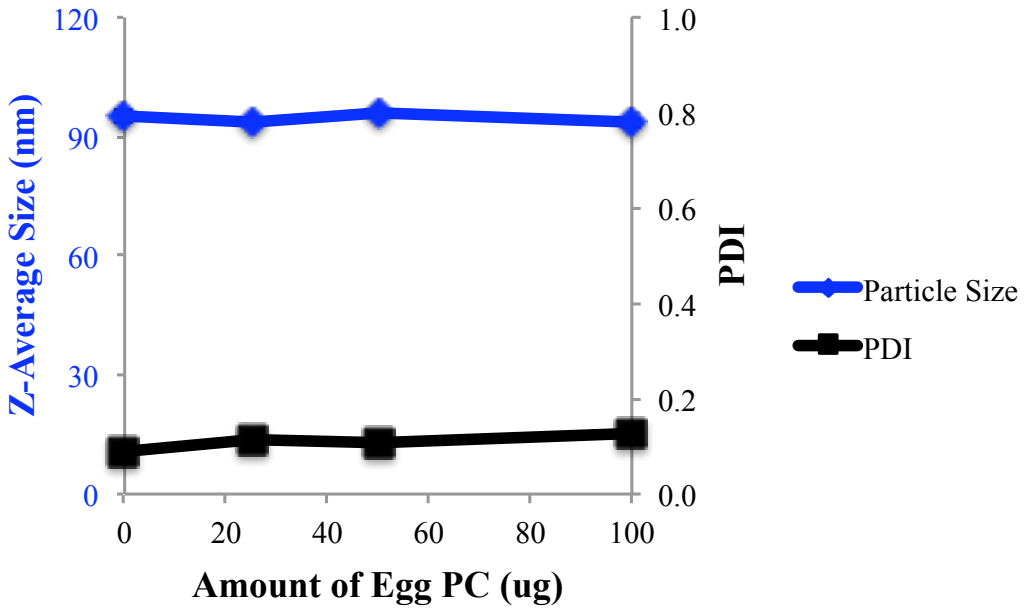

Figure 2.3. Effect of EGG PC concentration on nanoparticle size and polydispersity index (PDI)

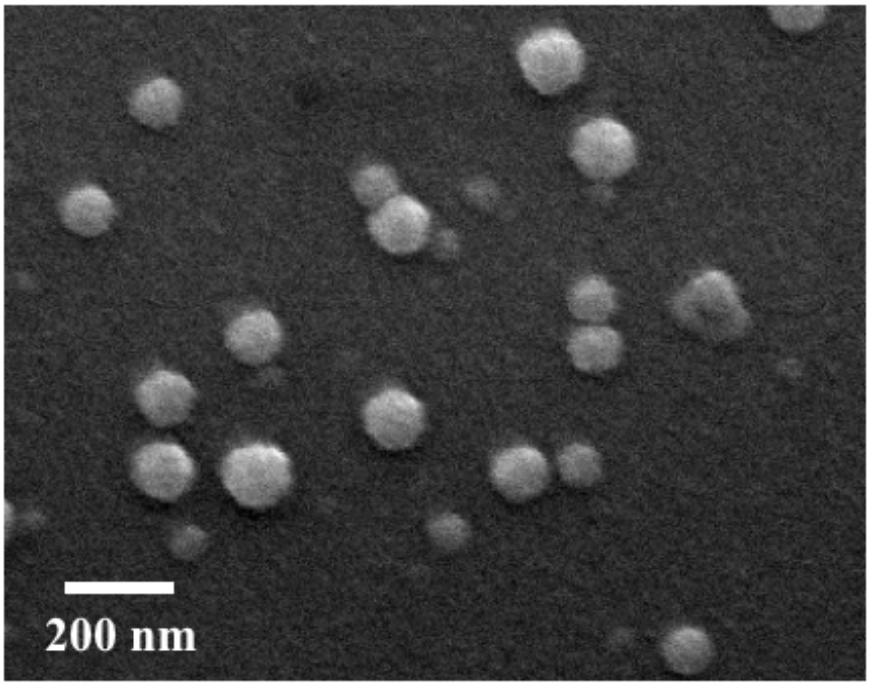

Figure 2.4. The SEM image shows particle morphology of hybrid nanoparticles with Egg PC as the lipid.

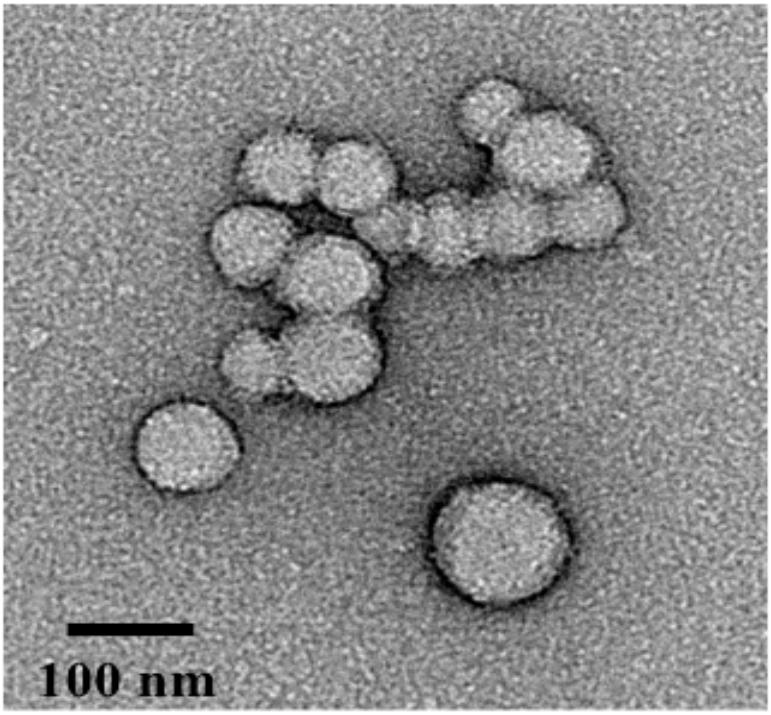

Figure 2.5. The TEM micrograph shows the internal structure of the hybrid nanoparticles with Egg PC as the lipid.

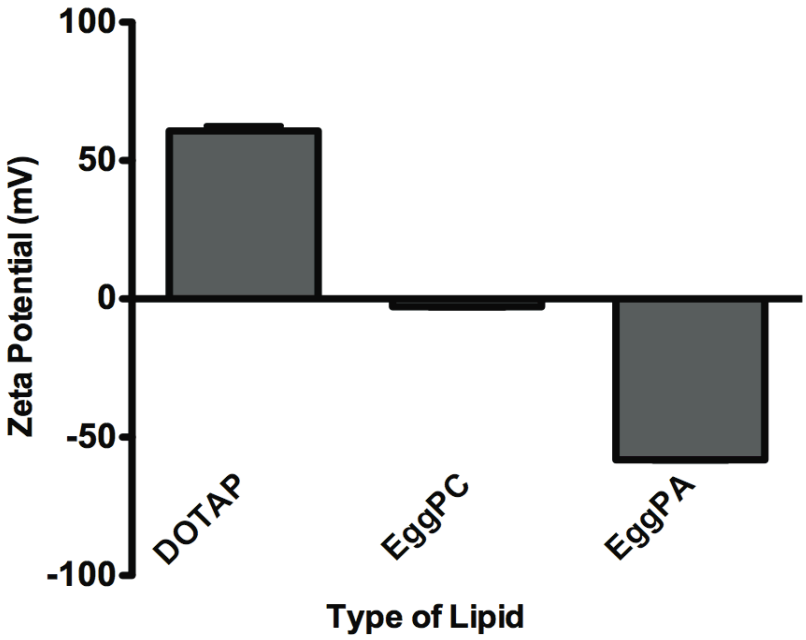

Figure 2.6. Zeta potential of control lipid vesicle solutions show that each lipid has a different surface charge.

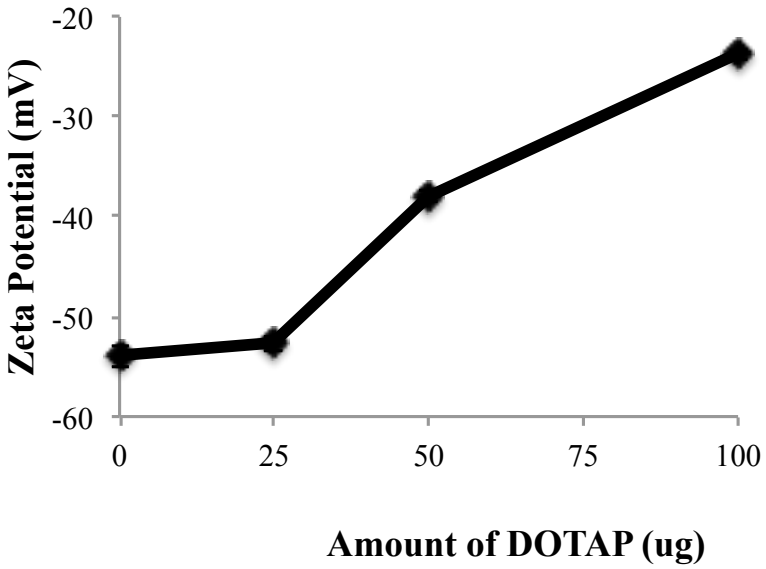

Figure 2.7. Effect of DOTAP concentration on zeta potential.

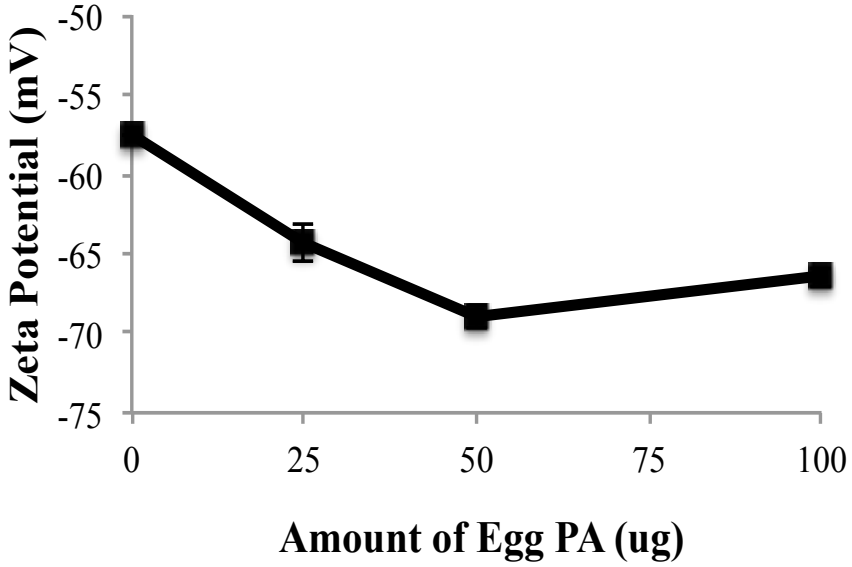

Figure 2.8. Effect of EGG PA concentration on zeta potential.

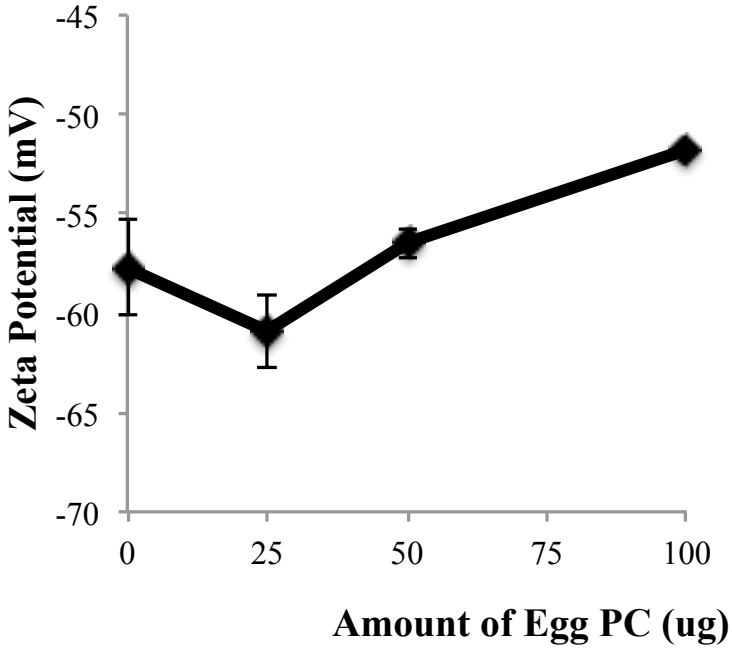

Figure 2.9. Effect of EGG PC concentration on zeta potential.

2.5 Conclusions and Outlook

The hybrid nanoparticle system is a robust platform for drug delivery because the particle size can be maintained at ~100 nm, and the surface charge can be modified with lipid concentration in an addition step of the fabrication process. Without lipids, the nanoparticle has a highly negative surface charge due to the carboxy group of the DSPE-PEG. Surface charge can be easily tuned by choosing the appropriate lipid type and by changing the lipid concentration. Depending on where the drug needs to be delivered, nanoparticles with a positive surface charge can enter cells through clathrin-mediated endocytosis, which is relatively quick while negatively charged nanoparticles internalize slower due to the negatively charged cell membranes. The particle size is an important component because nanoformulations can provide more improved drug release profiles and pharmacokinetic properties. With the sonication method of nanoparticle fabrication, the particles have a low polydispersity index, which is indicative of a narrow size distribution. The particles produced from this method have a spherical morphology according to the SEM data. This particle shape may have an impact on release kinetics as well as biodistribution. These results show that the hybrid nanoparticle platform can be tuned to have different surface charge. The versatility and the ease to apply surface charge modifications for this drug delivery system can be useful for targeting specific tissues and cells in different disease states. The fabrication process is reliable and produces particles with a polymer core and a lipid shell, which was confirmed by the TEM results. Hydrophobic drugs can be encapsulated in the polymeric core while lipophilic drugs can be encapsulated in the lipid shell. This hybrid nanoparticle system

proves useful as a way to deliver multiple drugs for combination therapy, which can reduce drug resistance with chemotherapeutic agents for example. These results show that the hybrid nanoparticle platform can be tuned to have different surface charge. The versatility and the ease to apply surface charge modifications for this drug delivery system can be useful for targeting specific tissues and cells in different disease states.

2.6 References

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Chapter 3: Combinatorial Drug Delivery with Lipid-Polymer Hybrid Nanoparticles

3.1 Introduction

Cisplatin, cisdiamine dichloroplatinum (II), is a square planar complex comprised of two amine groups and two chloride ions in a *cis* configuration around the metal center [1]. This compound is known for its antibacterial and cytotoxic capacity. Cisplatin works by targeting the DNA. The mode of action is that the molecule covalently binds to the DNA and in the process it distorts the double helix structure, which leads to cell death by apoptosis [1]. However, this mode of action is not a selective one and that limits the amount of cisplatin that can be dosed because of cytotoxicity to the patient. The dose-limiting toxicity as well as nephrotoxicity and neurotoxicity are side effects associated with the molecule [2]. Cisplatin has chemical properties that make it challenging for drug formulation. The solubility is low in water and it is insoluble in organic solvents. During the fabrication process, this lack of solubility would prevent effective drug encapsulation in a polymeric nanoparticle that contains a hydrophobic core. Low drug loading would lead to low blood levels and an inadequate therapeutic effect. The strategy is to improve the hydrophobicity and the organic solubility in order to effectively conjugate or encapsulate into a drug delivery system. In the literature, Cai et. al. developed a hyaluronan – cisplatin conjugate [3]. Another approach is to synthesize cisplatin with stearic hydrazide groups in the amine positions of the molecule in order to increase the lipophilicity. Chemically modifying cisplatin into bis(2-stearoylhydrazinyl)platinum(II) chloride (Pt-lipid) allows the compound to be loaded into a hybrid nanoparticle. It also has solubility in organic solvents, such as THF.

Camptothecin (CPT) is another widely used anticancer chemotherapeutic agent. It comes from the wood, bark, and fruit of the tree *Camptotheca acuminate* [4]. The mode

of action for CPT is that it selectively inhibits mammalian topoisomerase I, a DNA replication enzyme that is overly expressed in different tumor types including colon, ovarian, and esophageal carcinomas [4]. Topoisomerases are the enzymes that unwind the DNA. CPT confines the topoisomerase-I with DNA in a cleavage complex. This inhibition delays DNA replication, S-phase stops, apoptosis is initiated and leads to tumor cell death. In findings by Shao et. al. and Xia et. al., camptothecin can up-regulate pro-apoptotic proteins such as Fas, Fas ligand, Bax, and p21 [5,6]. CPT is efficacious in the lactone form, but when it is circulating in the body and exposed to physiological pH the lactone ring undergoes reversible hydrolysis leading to the more water-soluble and less active carboxylate form [7]. Human serum albumin has a high binding affinity for the carboxylate form, shifting the equilibrium in favor of the carboxylate form [6]. CPT is a S-phase specific drug that requires prolonged exposure to tumor sites in order to be effective. The drug has poor water solubility, poor in vivo stability of the active form, and toxicity [7]. In order to overcome these challenges, formulation strategies such as polymeric nanoparticles and liposomes have been employed.

Multidrug resistance is a common problem with cancer therapy and is an issue because of the multiple mechanisms that are accessible within the body. Multidrug resistance can be caused by a decrease in cytotoxicity of the drug to the cancer cells due to increased metabolism, reactions with increased levels of intracellular nucleophiles like glutathione, repair of drug-induced damage to DNA, and over expression of membrane-bound transporters, such as P-glycoprotein, that lower intracellular levels of the drug [8,9]. Combination therapy is a way to address some of these issues. Combination can mean co-administering multiple drugs in different delivery vehicles or multiple drugs

contained within a single delivery vehicle. Combination therapy has been used to treat malaria, HIV/AIDS, and cancer [10]. For cancer treatment, the combination of chemo-agents affects different targets and displays different toxicity profiles, which can improve drug efficacy or have comparable drug efficacy and reduced toxicity. Such examples of combination chemotherapy include the use of anthracycline daunorubicin, a DNA intercalator, with ara-C, a DNA polymerase inhibitor, for acute nonlymphocytic leukemia [10]. This combination of drugs interferes with DNA repair and DNA synthesis. Another example is for the treatment of colorectal cancer. Leucovorin is administered prior to 5-fluorouracil in order to enhance the binding and the ability to block the action of thymidilate synthetase in order to prevent DNA synthesis and repair.

In this work, the approach of chemical modification was used in order to improve the pharmaceutical properties of cisplatin, so that it would be advantageous for the drug delivery system. The hybrid nanoparticle fabrication process can allow for loading of multiple drugs within the single delivery vehicle. A cisplatin derivative and CPT were both loaded in lipid-polymer hybrid nanoparticles, where CPT is encapsulated in the polymeric core and the cisplatin derivative makes up the lipid shell, in order to deliver both drugs as a combination therapy. With both drugs co-delivered from the same nanoparticle system, an increase in potency is seen in the cytotoxicity results, the particle size is smaller, there is a decreased in burst release in vitro, and there is increased drug loading capacity. This combinatorial drug delivery in lipid-polymer hybrid nanoparticles can provide a functional way to deliver chemotherapeutic agents while avoiding the problem of multidrug resistance.

3.2 Materials and Methods

Hydrogenated L-α-phosphatidylcholine (Egg-PC) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (ammonium salt) (DSPE-PEG) were purchased from Avanti Polar Lipids, Inc. (Alabaster, AL). Ester-terminated poly(DL-lactic-*co*-glycolic acid) (PLGA) (inherent viscosity = 0.82 dL/g) was obtained from LACTEL Absorbable Polymers (Pelham, AL). (S)-(+)-Camptothecin (CPT) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan.). Stearic hydrazide was purchased from Tokyo Kasei Kogyo Co. Ltd. and used directly. Potassium tetrachloroplatinate (II), acetonitrile (ACN), tetrahydrofuran (THF), and all other chemicals used herein were purchased from Sigma-Aldrich Co. and used without further purification.

3.2.1 Preparation of bis(2-stearoylhydraziny)platinum (II) chloride (BSPC, Pt-lipid)

Bis(2-stearoylhydrazinyl)platinum(II) chloride (Pt-lipid) was prepared according to an earlier unpublished procedure from Aryal et al. Pt-lipid was synthesized in a biphasic solvent at room temperature. In a typical experiment, 10 mg (0.024 mmol) of potassium tetrachloroplatinate (II) was dissolved in 2 mL 0.05 M HCl and was reacted with 14.38 mg (0.048 mmol) of stearic hydrazide dissolved in 2mL of methylene chloride under vigorous stirring. After three days of reaction under vigorous agitation, the red color of the aqueous layer disappeared. Subsequently the organic phase becomes yellow colored. The organic phase was collected and precipitated in ether. Finally, the product was purified by column chromatography (5% methanol in chloroform) and thin layer

chromatography (3 % methanol in chloroform) product Rf= 0.44. ^{195}Pt NMR spectra were recorded in $CDCl_3$ using a Varian Mercury 400 MHz spectrometer. For ^{195}Pt NMR measurement, the shift in Pt-lipid resonance was measured with respect to the standard saturated solution of potassiumtetrachloro palatinate (II) in 0.05 M HCl containing 10% of D_2O. Samples were measured at the spectral width of 21615.8 Hz with spectral frequency of 107.22 MHz within a 200 ppm offset. Electrospray ionization mass spectrometry (ESI-MS, Thermo LCQdeca spectrometer) was used to determine the mass of the compound. ESI-MS (negative): m/z: 861.08 $[M-H]^-$, 896.94 $[M+Cl]^-$, 825.33 $[M-HCl-H]^-$. ^{195}Pt NMR δ ppm; -1578.0 (standard), -1377.2 (product).

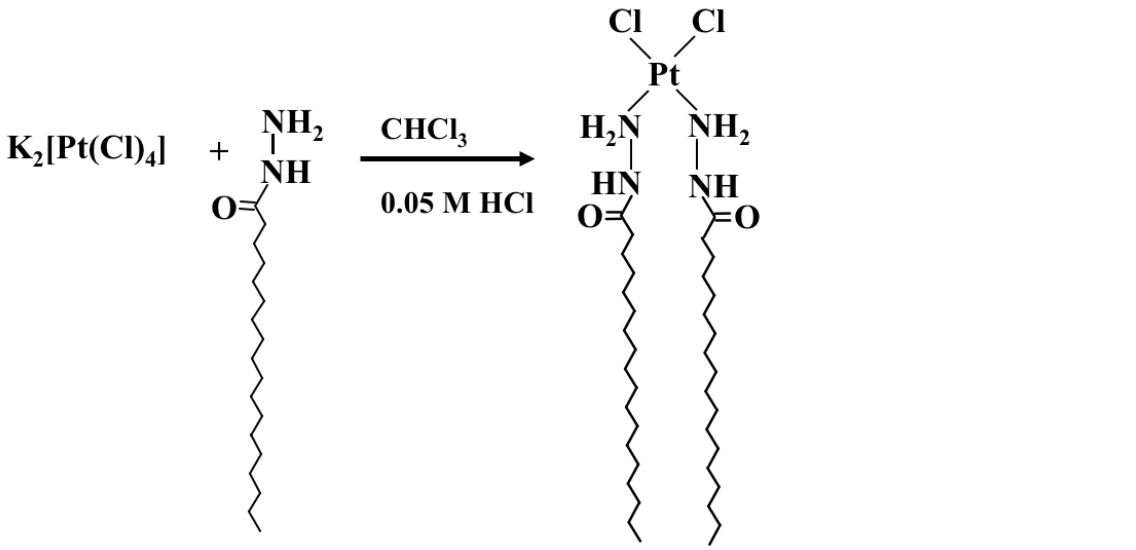

Figure 3.1. The synthetic scheme for bis(2-stearoylhydraziny)platinum (II) chloride (Pt-lipid).

3.2.2 Lipid-Polymer Hybrid Nanoparticle Synthesis

In a typical preparation, 200 μg of a platinum-lipid (Pt-lipid) solution in tetrahydrofuran (THF) was placed in a glass vial and the THF was evaporated using nitrogen gas. After the solvent has been dried off, 260 μL of a 1 mg/mL DSPE-PEG solution in 4% ethanol was added to the vial and the volume was adjusted to 2 mL with 4% ethanol. The sample was mixed while stirring at 80°C. In a separate glass vial, 100 μg of CPT in 100 uL of THF and 1 mg of PLGA in ACN was mixed and diluted to 1 mL with ACN. For the nanoprecipitation process, this 1 mg/mL polymer solution with 100 μg CPT was added dropwise to the Pt-lipid, DSPE-PEG sample on the heat plate. After that, 1 mL of water was added dropwise to the sample. The sample was then removed from the heat and placed on a stir plate at room temperature to stir for two hours in order to evaporate any leftover organic solvent. The solutions were washed 3 times with deionized water using a Millipore (Amicon Ultra) centrifuge filter with a molecular weight cutoff of 10 kDa. The samples were concentrated down to 1 mg of PLGA polymer to 1 mL of particle solution. Control samples were also prepared according to the procedure using EGG PC instead of Pt-lipid.

3.3 Characterization

Pt-lipid was characterized by ^{195}Pt-NMR to determine chemical identity. Thin layer chromatography (TLC) was used to purify the product and electrospray ionization mass spectrometry (ESI-MS) was used to confirm the molecular weight of the product. Particle size, polydispersity index (PDI), and zeta potential were measured to characterize the effect of dual drug encapsulation of camptothecin (CPT) and Pt-lipid on lipid-

polymer hybrid nanoparticle. Scanning electron microscopy (SEM) was employed to determine particle morphology and surface structure.

3.3.1 Pt-lipid

3.3.1.1 195Pt-NMR

^{195}Pt NMR spectra were recorded in deuterated chloroform ($CDCl_3$) using a Varian Mercury 400 MHz spectrometer. For ^{195}Pt NMR measurement, the shift in Pt-lipid resonance was measured with respect to the standard saturated solution of potassiumtetrachloro palatinate (II) in 0.05 M HCl containing 10% of D_2O. Samples were measured at the spectral width of 21615.8 Hz with spectral frequency of 107.22 MHz within a 200 ppm offset. ^{195}Pt NMR δ ppm; -1578.0 (standard), -1377.2 (product).

3.3.1.2 TLC (Rf)

The product was purified by column chromatography (5% methanol in chloroform) and by thin layer chromatography (3 % methanol in chloroform) with the product Rf= 0.44.

3.3.1.3 ESI-MS

Electrospray ionization mass spectrometry (ESI-MS, Thermo LCQdeca spectrometer) was used to determine the mass of the compound. ESI-MS (negative): m/z: 861.08 $[M-H]^-$, 896.94 $[M+Cl]^-$, 825.33 $[M-HCl-H]^-$.

3.3.2 Lipid-Polymer Hybrid Nanoparticles

3.3.2.1 Particle Size and Polydispersity Index (PDI)

Particle size measurements were performed by using dynamic light scattering (DLS) technique (Malvern Zetasizer, ZEN 3600). Three subruns were carried out per measurement and the average values were taken.

3.3.2.2 Zeta Potential

Zeta potential measurements were taken using the Malvern Zetasizer (ZEN 3600) in which the electrophoretic mobility on the surface of the nanostructures was measured. The measurements were carried out at room temperature with the backscatter angle of 173°. Three subruns were carried out per measurement, and the average values were taken.

3.3.2.3 Microscopic Analysis by SEM

Scanning electron microscopy was the technique used to look at morphology and surface structure of the hybrid nanoparticles. Samples for SEM were prepared by dropping 5 mL of a nanoparticle solution onto a polished silicon wafer. After drying the droplet at room temperature overnight, the sample was coated with chromium and then imaged.

3.4 Drug Loading

The initial amounts of both drugs contained within the lipid-polymer hybrid nanoparticles were assessed in order to determine encapsulation efficiency and a starting point for in vitro release studies. The initial camptothecin content was analyzed by UV-

Vis spectroscopy while the initial Pt-lipid content was measured by inductively coupled plasma analysis.

3.4.1 UV-Vis Spectroscopy

Lipid-polymer hybrid nanoparticle samples containing CPT were lyophilized and the remaining solids were dissolved in tetrahydrofuran (THF). Samples were analyzed with the UV-Vis spectrophotometer (TECAN, Infinite M200) using an absorbance wavelength of 362 nm.

3.4.2 Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES)

Samples with Pt-lipid were measured using the ICP-OES (Perkin Elmer, Optima 3000XL converted to Dual View) technique. Ytrium was used as an internal standard.

3.4.3 Reversed-Phase High-Performance Liquid Chromatography (RP-HPLC)

Drug loading for the samples used for the cytotoxicity study was determined using a C18 column with an Agilent Series 1100 system. The mobile phase was 60:40 (v/v) acetonitrile:water and the column temperature was set to 40°C.

3.5 In Vitro Drug Release

To measure the release profile of CPT from the lipid-polymer hybrid nanoparticles, the dialysis technique was used (10 kDa molecular weight cut off). Samples were dialyzed against 2L of pH 7.4 phosphate buffered saline (PBS) at 37°C. At

each time point, samples from three mini dialysis units were collected separately for drug quantitation by UV-Vis spectroscopy.

To measure the release profile of Pt-lipid from the lipid-polymer hybrid nanoparticles, the dialysis technique was used (12-14 kDa molecular weight cut off). Each formulation was dialyzed against 25 mL of pH 7.4 PBS at 37°C. At each timepoint, 3 mL of dialysis media was removed and collected, and 3 mL of fresh PBS was added. The samples were analyzed by ICP-OES to determine platinum metal content.

3.6 In Vitro Cytotoxicity

Cell viability was performed according to previous procedures published by Aryal et. al [11]. Cytotoxicity of the lipid-polymer hybrid nanoparticles was assessed against A2780 human ovarian carcinoma cell line using the (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. First, A2780 human ovarian carcinoma cells were seeded (2×10^4) in 96-well plates and incubated for 24 hours. Next, the medium was replaced with 150 μL of fresh medium and incubated with 50 μL of lipid-hybrid nanoparticle formulations for four hours. Then the excess nanoparticles were removed, and cells were washed three times with fresh buffer followed by the addition of fresh medium. The plates were then incubated for 72 hours and measured by MTT reagent following a protocol provided by the manufacturer. Fresh cell media and nanoparticles prepared with EGG PC were used as negative controls. Free drug at various concentrations was used as positive controls.

3.7 Results and Discussion

In previous unpublished works done by Aryal et. al., the synthetic approach was taken due to the ease of the coordination reaction with the potassium salt of the platinum chloride, potassium tetrachloroplatinate (II). In a biphasic solvent, Pt (II) is attached to the acyl chains and extracted into the organic solvent. During this extraction step, the inorganic salt of Pt (II) is converted to the organo platinum (II) complex (bis(2-stearoylhydraziny)platinum (II) chloride) (Pt-lipid). This formed Pt-lipid complex has decent solubility in organic solvent with a quantitative yield, ~ 75%. For the Pt-lipid, characterization was done by nuclear magnetic resonance to determine the chemical identity of the molecule after synthesis. Figure 3.2 shows a single signal for the standard at δ -1578.0 ppm. When coordinated with stearic hydrazide the chemical shift moves upfield at δ -1377.2 ppm. The *cis* configuration was confirmed by using the Kurnakov's test. The sample was treated with thiourea, which resulted in the formation of a yellow precipitate followed by crystallization. Yellow colored crystals shaped like needles were formed. The Pt-lipid complex was further confirmed by ESI-MS by determining the mass of the compound. The mass spectral data, shown in Figure 3.3, are in agreement with the calculated values and display the proper isotopic mass distribution patterns.

Camptothecin and cisplatin are S-phase chemoagents that disrupt DNA synthesis, which ultimately leads to cell apoptosis [1,4]. A convenient and elegant way to combine both modes of action from each drug is to formulate them together in a single lipid-polymer hybrid nanoparticle delivery vehicle. By delivering them together an increase in the therapeutic effect could be translated to further reduce cancer cell viability. The lipid-polymer hybrid nanoparticle platform is robust system in which the hydrophobic CPT can

be encapsulated inside the PLGA polymeric core and the lipophilic Pt-lipid complex can comprise the lipid shell with the Platinum as the head group. Three sets of formulations were fabricated: control nanoparticles containing only CPT, control nanoparticles containing only the Pt-lipid complex, and a combination nanoparticle formulation that contains both drugs. Physical characterization was done on these nanoparticles to determine particle size, zeta potential, polydispersity index, and morphology. As seen in Figure 3.4 and Table 3.1, particle size for the CPT formulation was 65 nm and the Platinum loaded one was 80 nm. When the two drugs are dually loaded in the system, the particle size was 61 nm. The combination particle size reflects closely with the CPT control nanoparticles. The polydispersity index values are indicative of homogeneous distribution of particles. Table 3.1 shows the surface charge values for each of the formulations, with the CPT control nanoparticles having the most negative charge (- 72 mV). The combination nanoparticles have a similar surface charge to that of the Pt-lipid control nanoparticles, both having a zeta potential ~ - 60 mV. According to Figure 3.5, the morphology for the combination hybrid nanoparticles was found to be spherical.

Drug loading percentage was determined for each drug from the combination nanoparticles. As seen in Figure 3.6, the control CPT nanoparticles had a 1.7% percent loading and control Pt-lipid nanoparticles had 0.6% Platinum loading, while the combination particles had 1.2% CPT and 0.9% Platinum drug loading. The combination formulation has a decrease in the CPT loaded content, but shows an increase in the Platinum loading. One possible explanation could be that the lipid portion of the Platinum complex is also incorporated into the polymeric core, which would decrease available space for CPT to reside in the core.

In vitro release studies were conducted and it is shown in Figure 3.9 that the CPT releases from the combination hybrid nanoparticle faster than the Platinum. The results show that there is minimal difference on release profiles when comparing the system loaded with one drug as opposed to dually loaded. Adding another drug does not significantly affect the release profile. This information suggests that the core does not affect hydrolysis of the shell. Within 72 hours, 100% of the CPT was released while only 55% of the Platinum was released. This could be an indication that Platinum release may last longer from the hybrid nanoparticles than 72 hours, but future studies would need to be conducted to confirm that. In vitro cytotoxicity was also examined with the lipid-polymer hybrid combination formulations. In Figure 3.10, the combination formulation shows that it can reduce ovarian cancer cell viability. Future studies need to be conducted where the relative cell viability is tested and compared against the free drug, a mixture of both free drugs, the single drug loaded hybrid nanoparticles, a cocktail mixture of the single drug loaded hybrid nanoparticles, and the combination nanoparticle formulation in order to determine if there is synergism in the dual loaded system.

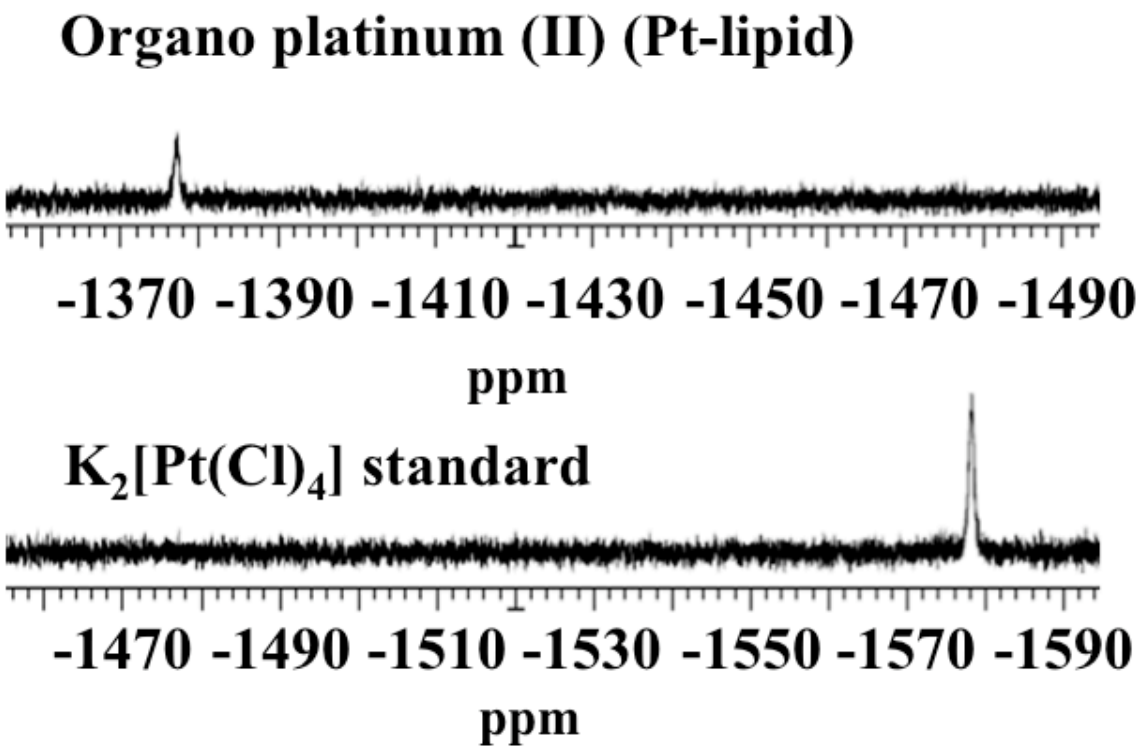

Figure 3.2. ^{195}Pt NMR spectra show the chemical shift for the prepared Pt-lipid compound is different from that of the platinum standard, which indicates that a new chemical entity was synthesized.

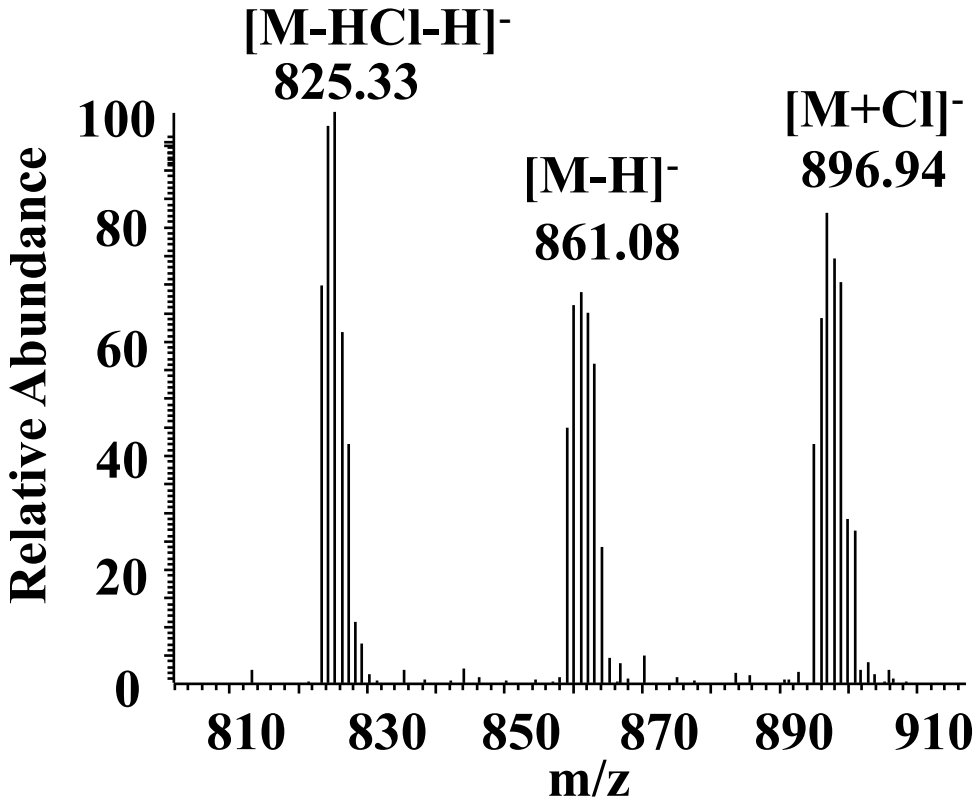

Figure 3.3. Electrospray ionization mass spectrometry shows the mass to charge ratios of the Pt-lipid complex.

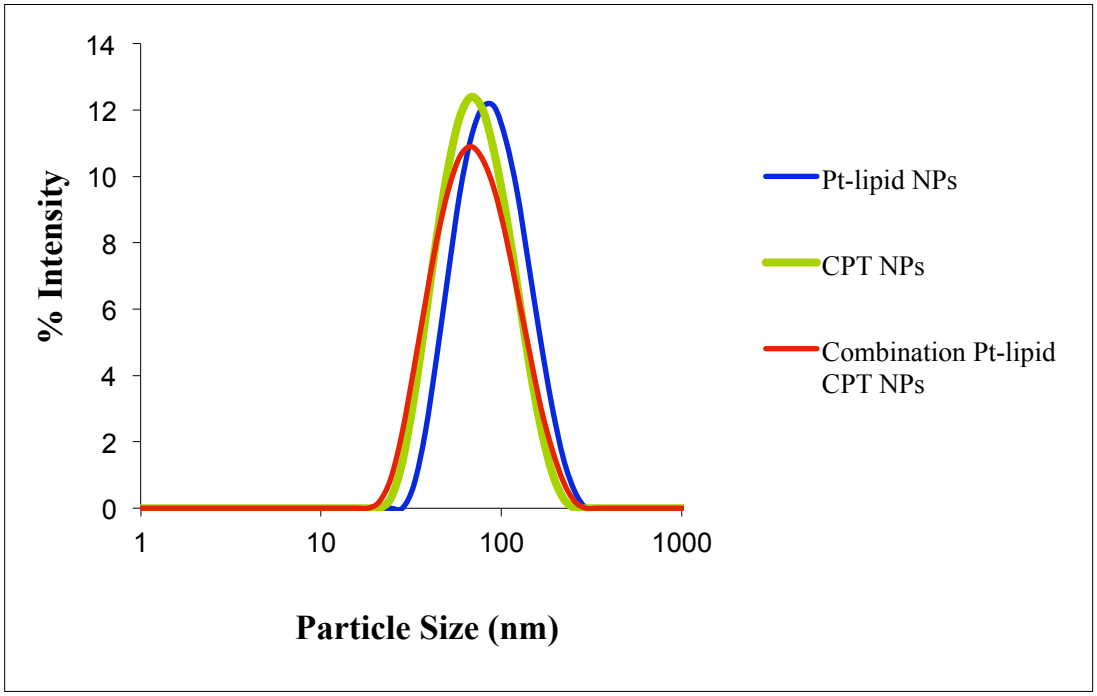

Figure 3.4. Dynamic light scattering measurements of the drug nanoparticles show similar particle size for the three types of hybrid formulations.

Formulation	Particle Size (nm)	Zeta Potential (mV)	PDI
Pt-lipid nanoparticles	80.1 ± 0.8	-59.8 ± 0.5	0.219 ± 0.008
CPT nanoparticles	64.9 ± 0.3	-72.1 ± 2.0	0.188 ± 0.008
Pt-lipid CPT combination nanoparticles	61.1 ± 0.1	-61.6 ± 0.4	0.255 ± 0.002

Table 3.1. Table shows the average particle size, zeta potentials, and PDI for each of the nanoparticle formulations.

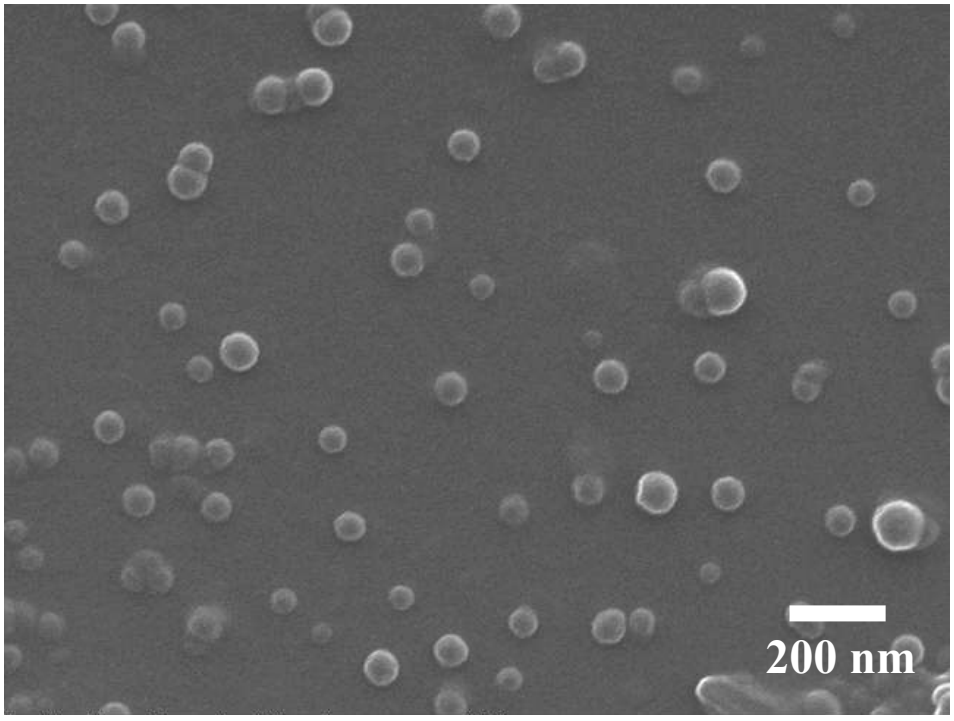

Figure 3.5. This is a representative SEM image of the combinatorial nanoparticles.

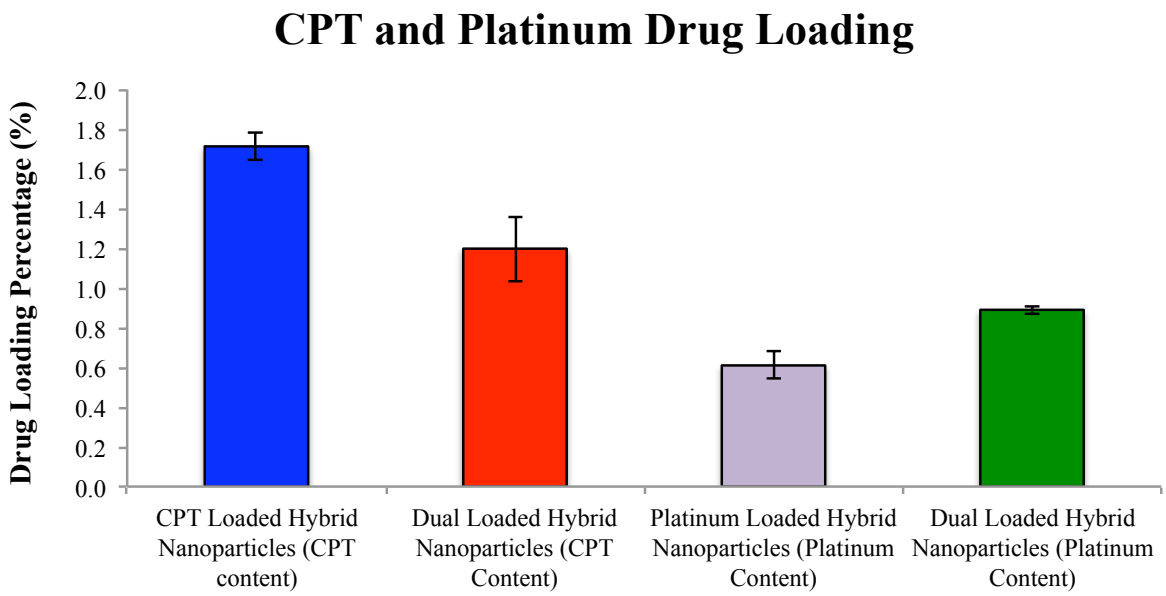

Figure 3.6. This graph shows the drug loading results for both camptothecin and Platinum separately as control nanoparticles and dually loaded in combination nanoparticles.

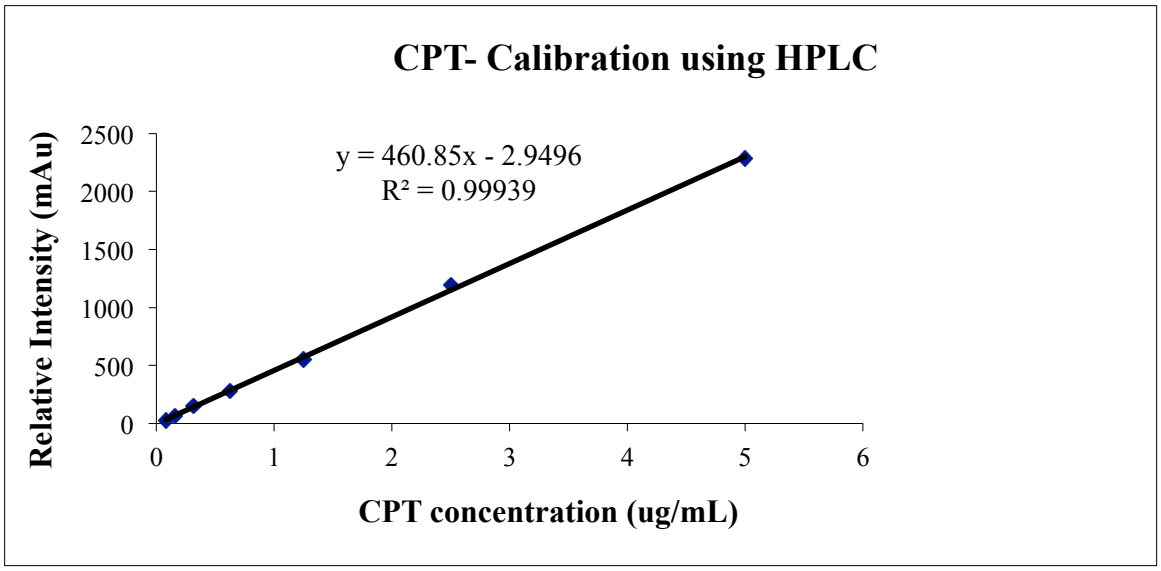

Figure 3.7. This calibration plot by HPLC was used to determine drug loading for CPT in the cytotoxicity samples because the drug levels were too low to be detected by UV-Vis spectroscopy.

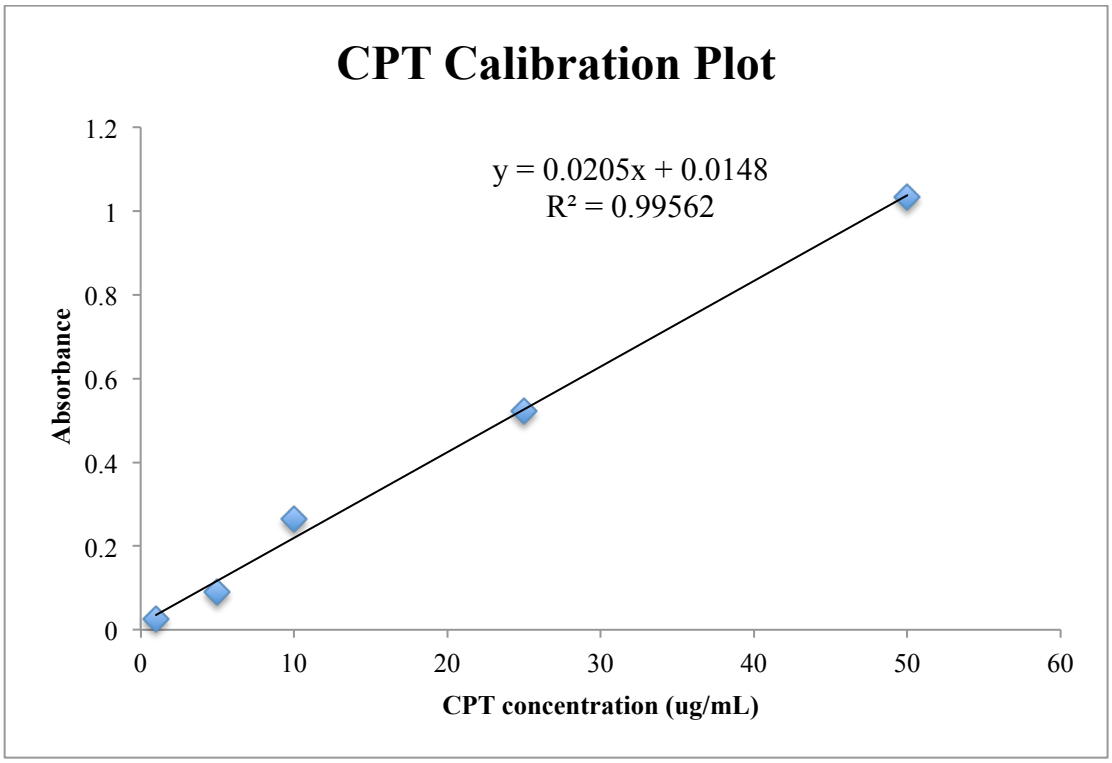

Figure 3.8. UV-Vis spectroscopy was used to determine CPT content during the in vitro release studies.

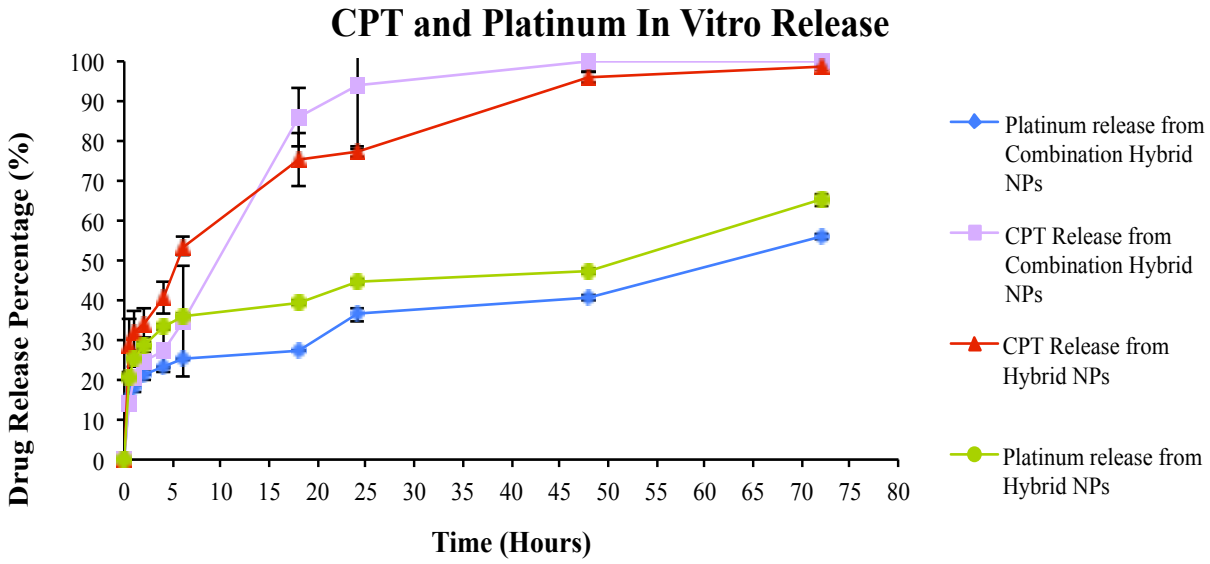

Figure 3.9. The in vitro release profile compares the drug release of CPT and of Platinum from the combination hybrid nanoparticles in which the CPT releases faster after 4 hours. It also shows that the addition of another drug to the hybrid nanoparticle system does not affect overall drug release profile as compared with control single drug loaded formulations.

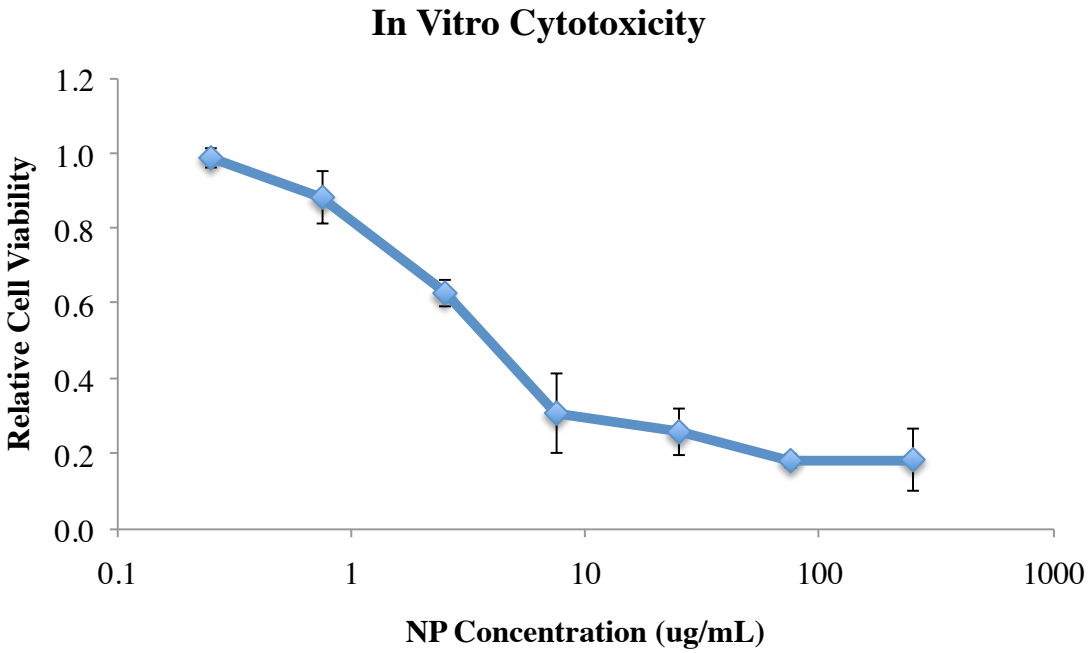

Figure 3.10. The cytotoxicity results show that the combination formulation is capable of reducing cell viability in ovarian cancer cells.

3.8 Conclusions and Outlook

The current course of cancer treatment falls short because of problems associated with nonspecific systemic distribution leading to cytotoxicity of healthy cells, poor circulation half-life, instability in the bloodstream causing decreases in efficacy, insufficient drug concentrations at the desired sites, and multidrug resistance. This work provides an approach to address the aforementioned concerns. By combining multiple drugs into a single delivery system, which can be functionalized to target specific tissues and cells, the drugs could have the opportunity to reach the targeted sites with the correct mass ratios and the possibility to induce therapeutic synergism.

3.9 References

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