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Synthesis and Characterization of Nanoparticle-Coupled Proteins in Human Serum Albumin

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An Honors Thesis submitted in partial fulfillment of the requirements for Honors in Physics.

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Under the mentorship of Dr. Li Ma

Abstract

Recently, cancer has become an ever-growing issue and has led to many researchers attempt to unravel the mystery of the disease. This research has led to a promising field of treatment: nanotechnology-coupled pharmaceuticals. Nanoparticles act as a whole unit when in conjugation with other molecules and add to the carrier molecule, most often proteins, benefits the nanoparticles themselves possess. One such carrier protein that can be conjugated with nanoparticles is Human Serum Albumin (HSA). Albumin is of interest in cancer research for two reasons: it is native to the human vasculature so it does not elicit immunological reactions, and it has tumor specificity. HSA made its main debut in cancer treatment when it was used to encapsulate Paclitaxel, an FDA approved cancer drug, to improve the drug delivery capabilities to hypoxic tumor cores via poly-nitroxylated nanoparticles. The primary goal of this study was to modify nitroxyl-decorated human serum albumin to stabilize Paclitaxel in order to synthesize a spin-albumin-stabilized Paclitaxel nanoparticle that will act as a cancer carrying protein with improved targeting of hypoxic tumor cores for cancer drug delivery while also having the nanoparticles add additional therapeutic properties to the drug complex: namely acting as an antioxidant and vasodilator. Data obtained from High Pressure Liquid Homogenization indicated that Paclitaxel was successfully loaded into nitroxyl-decorated HSA and nanoparticles formed in the correct size range of 100-200nm. Electron Spin Resonance data and spectroscopy data also confirmed the loading of Paclitaxel and allowed for the quantification of the number of loaded drug molecules.

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Introduction

In recent years, caner has become an ever-growing issue in the eyes of the public and with such attention, has led to the focus of many medical and scientific researchers being shifted towards unravelling the mystery of the disease. Although there are many different types of cancer, all of them have certain characteristics at the cellular level which are indicative of cancerous cells. Such characteristics include having self-sufficient growth signals, an insensitivity to antigrowth signals, ability to invade tissues (and reach metastasis

in the invaded tissues), a limitless potential for replication, sustained angiogenesis, and an ability to evade apoptosis (Lodish et. al., 2000). Although cancers can become specific to a certain tissue or region, it is these similarities among the different types of cancer that have allowed researchers to made strides towards more effective treatments for cancer patients.

One such method of treatment that has gained much attention in the recent years of study is the use of nanotechnology-coupled pharmaceuticals. Nanoparticles, although nanoscopic in size, act as a whole unit when in conjugation with other molecules and add to the carrier molecule any benefits the nanoparticles themselves possess as part of their physical or chemical properties. Nanoparticles also have the ability to accumulate at sites of inflammation making them suitable for targeted drug delivery (Das et al., 2005). In the case of cancer treatment, the use of stable nitroxyl radicals (nitroxides) can be made into nanoparticles that provide the carrier protein with the added benefit of acting as an antioxidant. Theses antioxidant allow for the stabilization of harmful free radicals in the blood in addition to the vasodilation of the vasculature in which they are present. This expansion of the blood vessels has been demonstrated by Kaul et al. in the treatment of sickle cell adhesion using antioxidants. Similar usage of nitroxides with albumin has also led to observed decreases in the infarct size of hemorrhages in rats (Beaulieu et al., 1998).

The results of each of these studies not only depended on nitroxide nanoparticles, but also on the type of carrier protein the nitroxide groups were conjugated with. One such carrier protein is Human Serum Albumin (HSA). HSA is a monomeric protein found in human blood that transports hormones, fatty acids, and other compounds (Purcell et al., 2000). Its role as a natural transport protein makes it an ideal candidate for conjugation with nanoparticles. Since HSA is a component of the blood plasma, about 5%, it is naturally

soluble and therefore can act as a carrier for hydrophobic drugs and medicines. Recently, HSA has been combined with nitroxyl free radical groups to create spin-labeled albumin that will hopefully become the next step in effective cancer treatment (Miele et al., 2009).

In 1982, a cancer drug, Paclitaxel (Taxol), was approved by the Food and Drug Administration for the treatment of cancer. However, due to Taxol being relatively insoluble in the blood stream, new methods were employed to help the delivery of the drug. Such research resulted in the formation of a new cancer drug: Abraxane (Abraxane BioScience, 2006). Abraxane is an albumin-stabilized Paclitaxel nanoparticle that acts as a mitotic inhibitor. In 2005, this drug was also approved by the FDA for the treatment of cancer; however, there was still a need for improvement in the delivery of the cancer drug to the cancer cells as shown by Matsumura and Kataoka in their study of anticancer agent-incorporated polymer micelles (Matsumura and Kataoka, 2009). The cutting edge research that is focusing on improving the current cancer treatments has utilized a new cancer treating agent: Vaxol. This new drug is a combination of Paclitaxel and VACNO (Vascular Albumin with Caged Nitric Oxide, Polynitroxyl Albumin). The combination of the PNA with Paclitaxel allows for the treatment of mitotically rampant cancerous cells while also destabilizing the condensed core of malignant tumors via the nitroxide free radicals (Desai et al., 2006). However, the current research utilizing VACNO or PNA are all in animal models and future research is aiming to replicate those results in human studies.

Although Paclitaxel has shown much promise in its ability to improve cancer treatment, it still poses some difficulties to researchers attempting to use it for pharmaceutical purposes. Paclitaxel, as previously mentioned, is unstable in the blood stream and in fact, in most aqueous solutions, as was demonstrated in a study conducted by Amini-Fazl et al. using

multiple aqueous solvents (Amini-Fazl et al., 2013). Therefore, it is commonly dissolved in ethanol, a solvent which is dangerous to inject intravenously in high concentrations. How then does one resolve the issue of conjugating Paclitaxel with a protein found commonly throughout the blood stream? The answer lies within a High Pressure Homogenizer.

High Pressure Homogenization (HPH) has recently become a popular method for suspending hydrophobic and insoluble drugs in a carrier medium (Nanjwade et al., 2010). Published studies by Shelar et al. and Jacobs et al. have demonstrated how HPH can be used in tandem with other methods to fabricate isradipin and tarazepide nanosuspensions, both hydrophobic drugs, respectively (Shelar et al., 2013; Jacobs et al., 2000). The former of these studies also showed that this method allowed for more bioavailability of the synthesized drug; which was a major reason in choosing this method as the fabrication method for the drug-protein complex for this study.

Aims of Study

The primary goal of this study was to modify nitroxyl-decorated human serum albumin to stabilize Paclitaxel in order to synthesize a spin-albumin-stabilized Paclitaxel Nanoparticle that will act as a cancer carrying protein with improved targeting of hypoxic tumor cores for cancer drug delivery. The goal is to also have the nanoparticles add additional therapeutic properties to the drug complex: namely acting as an antioxidant and vasodilator. It is hypothesized that the synthetic complex will form as a tetramer comprised of four identical spin-labeled albumin monomers. In order to test if the synthesis was successful, numerous biochemical and physical analytical methods will be employed to quantify the carrier protein's size, structure, and properties. If the synthesis is a success, the next step would be to test the synthesized carrier protein in preliminary pharmaceuticals to be

used on animals in order to test for effectiveness in the reduction of malignant tumors and cancerous cells. The latter of the two goals for this study is time sensitive and also relies on the success of the first step of the project.

Materials and Methods

Chemicals

Human serum albumin and modified human serum albumin, or polynitroxylated human serum albumin were used at the protein carriers in this experiment. Paclitaxel (Figure 1) was used as the anti-cancer agent that was to be loaded into the aforementioned protein carriers. Chloroform and ethanol (9:1 v:v) were used as solvents for preparing Paclitaxel to be loaded while phosphate buffer (pH 7.4) was used as the solvent for HAS and PNA.

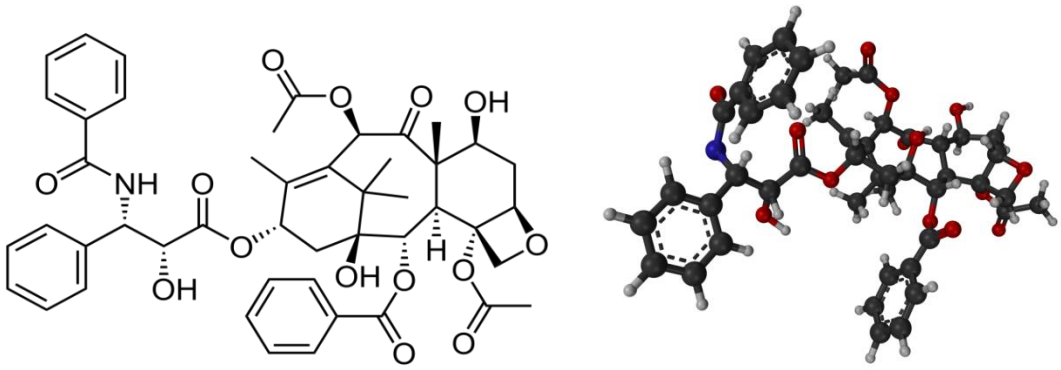


Figure 1. The structure of Paclitaxel.

Preparation of Human Serum Albumin

Human serum albumin (HSA), obtained from Baxter, was diluted from a 25% w/v stock solution to 5% w/v using 0.9% w/v aqueous sodium chloride. The stock HSA had stabilizers as well as fatty acids attached to it that were removed prior to protein modification. The stabilizers were removed by filtration using a Millipore peristaltic pump and a 30kDa cassette filter. This filtration technique separated the solution based on the differences in the molecular sizes of the protein versus the stabilizer allowing for the protein

to be isolated. Next, the HSA solution was treated with activated carbon in order to remove the fatty acids that were conjugated with the protein. The method used to carry out the treatment followed the guidelines published by Raymond Chen in *The Journal of Biological Chemistry* (1967). Finally, the HSA was filtered through a 0.22µm syringe filter and the solution's pH was adjusted to biological levels (pH=7.4). An aliquot of the purified HSA was kept to serve as a control during the analysis of the final synthesized product.

Drug Loading

In order to load Paclitaxel into HSA and PNA respectively, a high pressure liquid homogenizer (Nano DeBEE) was used. For each 20mL processing volume of the protein-drug solution at 10mg/ml concentration, 20mg of Paclitaxel was dissolved in 0.4mL of a 9:1 solution of chloroform and ethanol while 20mL of 1% w/v HSA or PNA was formed using a 5mM Phosphate buffer solution (PBS, pH 7.4). The two solutions were gently mixed together and then emulsified using high pressure liquid homogenization. The samples were homogenized for two cycle times at a pressure of 1500 psi followed by homogenization for two cycle times at 3000 psi, and finished with 8 cycles at 30,000 psi. Following homogenization, the final dispersion underwent rotary evaporation under reduced pressure (40°C, 15 min) in order to remove chloroform from the solution. Next, the nanoparticles were removed by centrifugation at 6000 rpm; however, it should be noted that lower rotational speeds may be needed depending on the state of the final dispersion in order to preserve the composition of the nanoparticles. Finally, the supernatants for each sample obtained by centrifugation were lyophilized and stored at -20°C until analysis.

Analysis of Synthesized Product

After the protein carrier complex is loaded with the cancer treatment drug and spin-labeled with nitroxides, numerous analytical techniques were used to verify the structure of the modified Taxol. The results of the analytical tests were used to quantify the level to which the synthesized product matched the hypothesized complex that was expected to form. The first analytical method that was employed was testing for electron spin resonance. This method used an induced magnetic field to identify the presence of any unpaired electrons in the complex by measuring the change in energy between the electrons with spins parallel to the magnetic field lines and those that are flipped anti-parallel when in resonance with microwave radiation. This analysis was only performed on modified albumin and modified albumin- paclitaxel since they are decorated with nitroxide groups which have unpaired electrons between nitrogen and oxygen atoms, whereas HSA does not have any free electrons and appears as EPR inactive. The concentration of free radicals was determined in the drug loaded sample and compared to the control sample (modified albumin) to ensure that the addition of Paclitaxel did not alter the free radical density of the initial PNA molecule.

Next, the lyophilized samples were reconstructed in distilled water and centrifuged to half volume through a 3kDa microfilter and the filtrate was collected in order to determine the concentration of unbound paclitaxel. To determine amount of bound and unbound paclitaxel, the retentate was then titrated with EtOH in order to precipitate any free proteins and then centrifuged once more (10 min, 13.4 krpm) in order to isolate the Paclitaxel-loaded proteins in the supernatant. Both the filtrates and supernatants had absorbance data collected and from them and a loading factor for the synthesized drug carrier was calculated by

observing the difference in absorption at 227nm, which is the absorption peak for Paclitaxel. (Zhao et al., 2010).

Results

High Pressure Homogenization (HPH)

As previously mentioned, High Pressure Homogenization (HPH) is one of the few methods that can combat the issues that arise when attempting to form drugs with molecules that are unstable or immiscible in aqueous and organic media. Since Paclitaxel falls into this category of immiscible drugs, HPH was used to load the drug into both HSA and PNA. The average sizes of these proteins before undergoing HPH and drug loading are listed in Table 1; these values were used as standards in order to determine if nanoparticles were successfully formed. The average molecular diameters of the control proteins as well as the drug loaded proteins after undergoing HPH are listed in Table 2. When compared to the pre-HPH sizes, it was confirmed that this method produced nanoparticles for both control proteins as well as both drug-loaded samples.

Table 1. Comprehensive physical data summary of control protein sizes, HSA and PNA, before undergoing high pressure liquid homogenization.

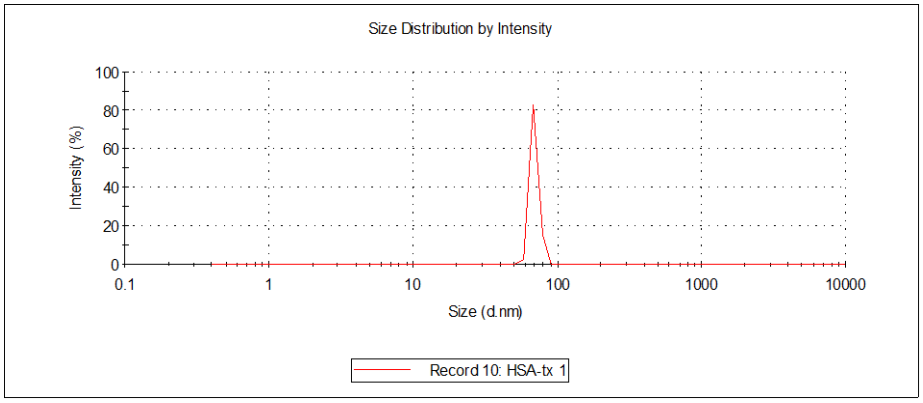
Sample Name	Z-Ave	PdI	Pk 1 Mean Int	Pk 2 Mean Int	Pk 3 Mean Int	Pk 1 Area Int	Pk 2 Area Int	Peak 3 Area Intensity
	d.nm		d.nm	d.nm	d.nm	%	%	%
PNA Original	11.26	0.224	12.57	2.623	0	94.5	5.5	0
HSA Original	30.24	0.651	100.2	8.495	0	73	27	0

Table 2. Comprehensive data summary of control protein sizes, HSA and PNA, along with experimental drug loaded protein sizes, HSA-PTX and PNA-PTX, after undergoing high pressure liquid homogenization.

Sample Name	Z-Ave	PdI	Pk 1 Mean Int	Pk 2 Mean Int	Pk 3 Mean Int	Pk 1 Area Int	Pk 2 Area Int	Peak 3 Area Intensity
	d.nm		d.nm	d.nm	d.nm	%	%	%
Free albumin	149.5	0.226	187.2	0	0	100	0	0
PNA	133.7	0.437	188.7	10.09	0	92.8	7.2	0
HSA-PTX	1765	1	69.46	0	0	100	0	0
PNA-PTX	375.9	0.388	204.1	0	0	100	0	0

Figure 2 shows the size distribution peaks versus peak intensity for the two drug loaded samples, HSA-PTX and PNA-PTX, respectively. However, these figures only show intensity data in the domain for nanoparticles and it should be noted that there were large absorption peaks outside of this range that were centrifuged out after the average diameters were measured, therefore the average molecular sizes in the table are skewed from the purified samples that were collected following the measurements.

(a)



(b)

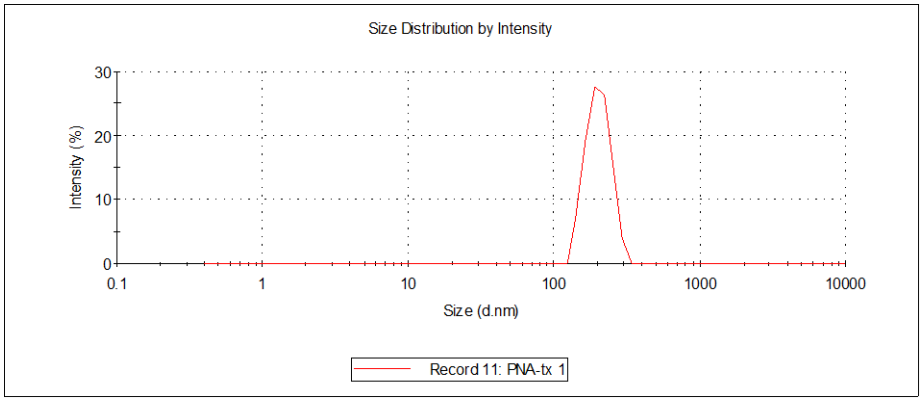


Figure 2. Size distribution by intensity of (a) drug-loaded HSA and (b) drug-loaded PNA synthesized via high pressure liquid homogenization before lyophilization.

In order to preserve the nanoparticles after homogenization the samples were freeze dried via lyophilization. Following lyophilization the average molecular sizes of the nanoparticles were measure once again (Table 3).

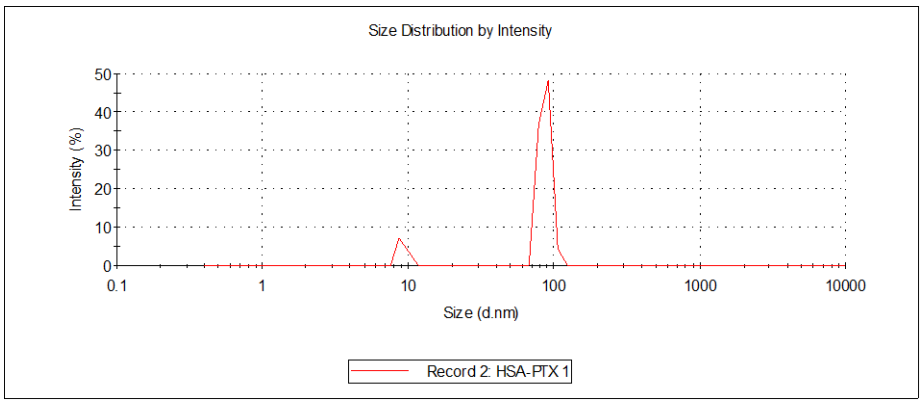
Table 3. Comprehensive data summary of control protein sizes, HSA and PNA, along with experimental drug loaded protein sizes, HSA-PTX and PNA-PTX, after undergoing lyophilization.

Sample Name	Z-Ave	PdI	Pk 1 Mean Int	Pk 2 Mean Int	Pk 3 Mean Int	Pk 1 Area Int	Pk 2 Area Int	Peak 3 Area Intensity
	d.nm		d.nm	d.nm	d.nm	%	%	%
Free HSA 1	124.4	0.328	175.9	0	0	100	0	0
HSA-PTX 1	804.2	0.716	86.81	9.169	0	89.1	10.9	0
Free PNA 1	180.9	0.284	10.1	82.77	0	55.1	44.9	0
PNA-PTX 1	719.5	0.787	59.63	7.34	0	82	18	0

Additionally, the two drug-loaded samples had their molecular diameters measured against their peak intensities after being lyophilized. It was observed that the freeze drying process resulted in the reduction of the average nanoparticle size in both drug-loaded samples (Figure 3). Similar to the skewing that occurred with the average molecule sizes before lyophilization, the same patterns in the data was observed in Table 3 when compared to the nanoparticle signals recorded for the drug loaded samples. Once again, this was due to the

size distribution by intensity only recording data over a set range whereas the comprehensive data in Table 3 averaged all molecule sizes present in the sample.

(a)



(b)

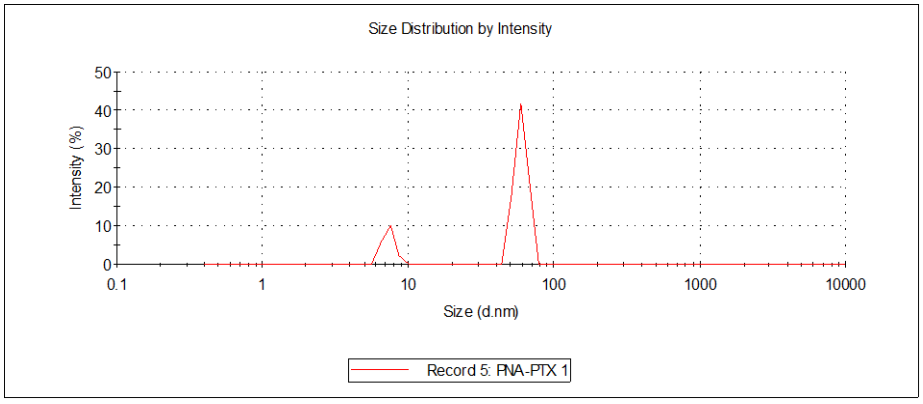


Figure 3. Size distribution by intensity of (a) drug-loaded HSA and (b) drug-loaded PNA synthesized via high pressure liquid homogenization after lyophilization.

Electron Spin Resonance (ESR)

The nitroxide density on the synthesized drug carrier was determined using Electron Spin Resonance (ESR). After a series dilution was performed in order to create a standard curve of concentration versus second integration values for stock PNA (100mg/mL), the second integration values for the reconstructed PNA and PNA-PTX nanoparticles were measured and converted to concentrations, as shown in Figure 4.

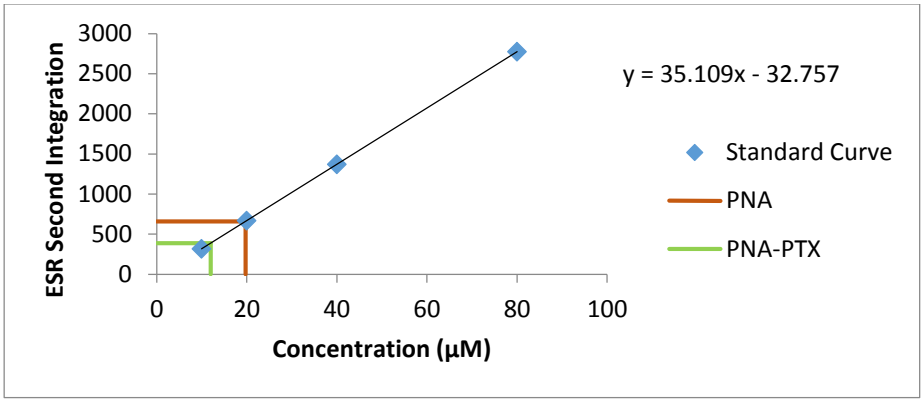


Figure 4. Standard curve produced by a series dilution of stock modified albumin of electron spin resonance second integration values versus concentration of modified albumin. The concentrations of reconstructed PNA and PNA-PTX are shown as intersections of their second integration values with the standard curve.

These values were then used along with dilution factors and absorbance values at 280nm in order to calculate the ratio of nitroxide free radicals on the surface of PNA and PNA-PTX. These ratios were then compared to the ratio present in the stock solution of modified albumin (Figure 5).

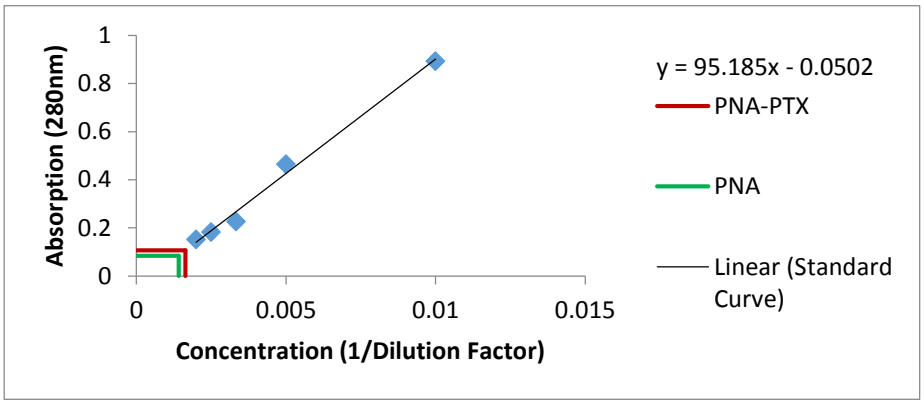


Figure 5. Standard curve produced by a series dilution of stock modified albumin of absorption values at 280nm versus concentration. The concentrations of reconstructed PNA and PNA-PTX are shown as intersections of their absorption values with the standard curve.

Absorbance Spectra

Once it was determined that the addition of Paclitaxel only slightly altered the surface composition of the free radicals, the number of loaded drug molecules were calculated as a ratio of milligrams of Paclitaxel to milligrams of PNA. This ratio was determined by

examining normalized absorption spectra for the drug-loaded samples and comparing them to the unloaded samples. The filtrates and supernatants from both controls and experimental proteins were analyzed via spectroscopy (Figures 6 and 7). Specifically, the difference in absorption peaks at 227nm was quantified, as this is the absorption wavelength for Paclitaxel, and used in conjugation with a standard curves for PNA absorbance at 280nm and Paclitaxel absorbance at 227nm (Table 4, Figure 5, and Figure 8).

(a) (b)

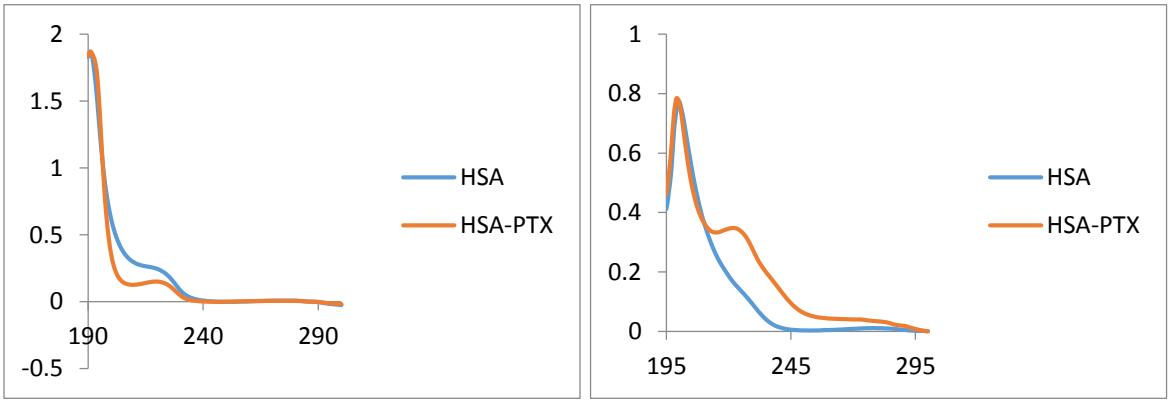


Figure 6. (a) Normalized absorption spectrums for HSA (4x dilution) and HSA-PTX (4x dilution) filtrates. (b) Normalized absorption spectrums for HSA ($1H_2O$:7EtOH and 4x dilution) and HSA-PTX ($1H_2O$:7EtOH and 4x dilution) supernatants.

(a) (b)

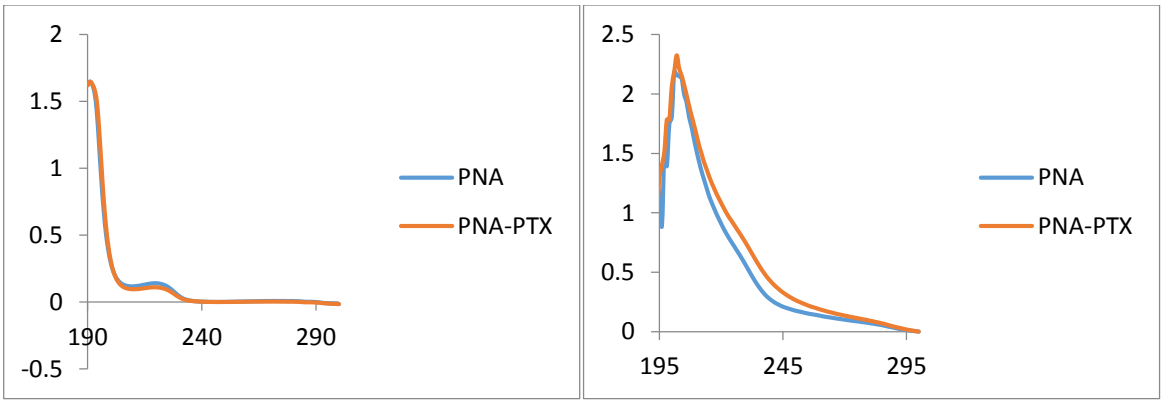


Figure 7. (a) Normalized absorption spectrums for PNA (4x dilution) and PNA-PTX (4x dilution) filtrates. (b) Normalized absorption spectrums for PNA ($1H_2O$:7EtOH and 5x dilution) and PNA-PTX ($1H_2O$:7EtOH and 4x dilution) supernatants.

Table 4. Differences in absorbance at 227nm for the filtrates and supernatants of the drug-loaded samples compared to their respective controls.

Compound	HSA/HSA-PTX Filtrate	HSA/HSA-PTX Supernatant	PNA/PNA-PTX Filtrate	PNA/PNA-PTX Supernatant
Absorption Difference at 227nm	-0.056956212	0.199381791	-0.019078095	0.124223441

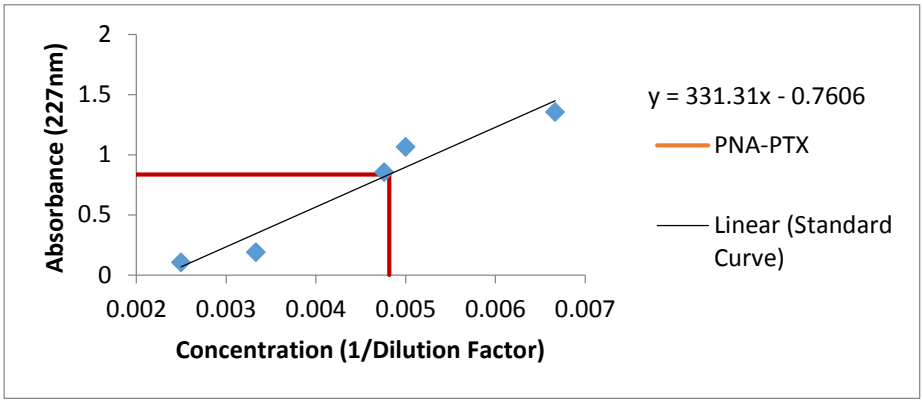


Figure 8. Standard curve produced by a series dilution of 9mM Paclitaxel of absorption values at 227nm versus concentration. The concentration of reconstructed PNA-PTX is shown as an intersection of its absorption values with the standard curve.

Discussion

Paclitaxel is an FDA approved cancer treatment that has been shown to have beneficial consequences for impacting the size of tumors in patients with cancer. Despite its potential benefits, Paclitaxel still had issues with respect to its ease of delivery that made treatment with the drug especially difficult. Paclitaxel's instability in aqueous solutions leads to low efficiency in intravenous injections and its solubility in ethyl alcohol is incompatible with the human bloodstream. As a result, much research has been conducted to come up with a potential answer to these drug delivery issues: carrier proteins.

Protein conjugation has made great strides in assuaging the difficulties of effective cancer treatment with Paclitaxel, such as increasing the stability and half-life of the drug in human vasculature (Kratz, 2008). However, despite the success protein conjugation has had

with making the drug more stable, it did not make the drug completely ideal. Most notable of the remaining issues with the drug was its ability to target and penetrate tumors. In 2009 Desai and coworkers attempted further understand the drugs targeting issue by studying the extent to which Paclitaxel conjugated albumin penetrated tumors located in the cerebral and cervical region of patients. What they found was that patients whose tumors had higher SPARC secretion had an increase in drug delivery efficiency. These results were concluded to be a consequence of SPARC's albumin-binging properties.

What then for the patients who do not have high SPARC expression to help improve drug targeting? According to the previously mentioned 2006 study conducted by Kaul et al., one of most effective antioxidants used in the study for inhibiting sickle cell adhesion was PNA. PNA was shown to result in no post-capillary blockage by adhered sickle cells as well as almost complete inhibition of said adhesion by PNA. Therefore it was hypothesized for this study that if Paclitaxel was loaded onto PNA instead of HSA, then the antioxidant nature of the protein carrier would target the hypoxic core of metastasized tumors, thus improving drug delivery efficiency for SPARC positive and SPARC negative patients alike.

In order to synthesize this new modified albumin drug carrier protein HPH was used so as to preserve the chemical structure of Paclitaxel in an otherwise unstable solvent. The size data obtained from the high pressure homogenizer before lyophilization indicated that the synthesized nanoparticles were of the correct size range (100-200nm) to be effective in medicinal use for both HSA and PNA. However, after lyophilization there was a noted decrease in the nanoparticle sizes for PNA. After preserving the samples by rapid freeze drying, the analytical steps of the project were then carried out.

One of the main draws of using PNA as a drug carrier versus HSA is that the free radical groups allow the protein to target and penetrate the hypoxic core of tumors. Therefore, it was important to verify that conjugating Paclitaxel with PNA did not interfere with the nitroxide groups on the protein's surface that provide the previously mentioned anti-tumor benefit. In order to determine if the drug loaded had any effect on the PNA's free radical surface density Electron Spin Resonance was used as the first analytical technique for determining the physical characteristics of the newly synthesized drug carrier protein complex. The samples of PNA-PTX and PNA had concentrations similar to samples produced for the standard curve based on their second integration values, therefore the sample's intensity peaks and absorption values at 280nm were used to calculate a ratio of nitroxides on the samples which were then compared to the known nitroxide density on the stock PNA from which the samples were made.

$$Free\ Radical\ Ratio(FRR) = \frac{(Dilution\ Factor\ for\ ESR)(ESR\ Secnod\ Integration\ Value)}{(Dilution\ Factor\ for\ Absorption)(Absorption\ at\ 280nm)} \quad (1)$$

$$\text{PNA: } FRR = \frac{(160)(317.7001)}{(.084455)(10)} = 60{,}188.28489 \quad (2)$$

$$\text{PNA-PTX: } FRR = \frac{(133.5406277)(387.89486)}{(.10634)(10)} = 48{,}711.41911 \quad (3)$$

$$\frac{PNA-PTX\ FRR}{PNA\ FRR} = \frac{48{,}711.41911}{60{,}188.28489} = 0.809 \quad (4)$$

Based on the data and above calculations (Equation 1-4), there was a 4:5 ratio of nitroxides on the reconstructed PNA versus the reconstructed PNA-PTX. These values suggest that the addition of Paclitaxel to the proteins slightly altered the number of free radicals that were originally present in each molecule by a 20% reduction. However, since the drug loaded protein and original sample are still comparable in free radical density, the vasodilation effects may still be present in both; however further research would be needed in order to

conclude this hypothesis. Also, it is not clear the mechanism of the reduction during the formation of nanoparticles.

Following EPR, the samples were centrifuged and filtered according to the methods listed above in order to isolate the bound Paclitaxel-protein complexes. Absorption spectra were then obtained for the filtrates and supernatants of all samples to be used in calculating the number of loaded drug molecules on each protein. The calculations in equations 5-9 were performed using multiple standard curves (Figures 5 and 8) as well as of the absorption difference at the wavelength 227nm, the absorbance peak for Paclitaxel.

$$Absorbance\ of\ \frac{4mg\ PNA-PTX}{10mL\ H_2O}\ at\ 227nm: 0.83573 \rightarrow 207.548x\ dilution\ factor \quad (5)$$

$$\frac{1}{207.548}\left(9 \cdot 10^{-3}\,\frac{mol}{L}\right)\left(\frac{853.906g}{1mol}\right)\left(\frac{1L}{1000mL}\right)\left(\frac{1000mg}{1g}\right) = 0.0362\,\frac{mg\ PTX}{mL\ H_2O} \quad (6)$$

$$Absorbance\ of\ \frac{4mg\ PNA-PTX}{10mL\ H_2O}\ at\ 280nm: 0.23319 \rightarrow 335.847x\ dilution\ factor \quad (7)$$

$$\frac{1}{335.847}\left(100\,\frac{mg\ PNA}{mL\ H_2O}\right) = 0.29775\,\frac{mg\ PNA}{mL\ H_2O} \quad (8)$$

$$Ratio\ of\ Converted\ Absorbances = \frac{\left(0.0362\frac{mg\ PTX}{mL\ H_2O}\right)}{\left(0.29775\frac{mg\ PNA}{mL\ H_2O}\right)} = 0.122\,\frac{mg\ PTX}{mg\ PNA} \quad (9)$$

It was determined that the modified HSA molecules had an average of 0.122mg of Paclitaxel per 1mg of PNA. The amount of loaded Paclitaxel molecules on PNA was comparable to the amount loaded to unmodified HSA in commercially available Paclitaxel (11.1%).

Overall Implications of Research

As previously mentioned, cancer has become a very "hot-button" issue with the public and thus has been the focus of many recent scientific research projects. This field of study is of particular interest because of new methods of drug delivery that offer promising results with respect to treating cancer patients. For example, it has recently been shown that

spin labeled albumin can increase blood flow while also increasing the permeability of the drug into tumors in mice (Sugawara et al, 2001). Therefore it was this project's goal to combine the tumor specific benefits of nitroxides with the protein carrier benefits of Paclitaxel loaded PNA to increase the efficiency of drug delivery to tumors in cancerous patients. If this project is a success, then the result would be an increased effectiveness in cancer treatment by incorporating the ability to break up the tightly packed core of tumors to an FDA approved cancer treatment drug. This added benefit, which stems from the nanoparticles being in a complex with Paclitaxel, ultimately would allow for the cancer treatment to be more effective in that it could deliver the drug into the core of the cancerous cell cluster instead of just to the surface. Also, the antioxidants would have the added benefit of intercepting harmful radicals in the body as well as alleviating hypertension and vascular blockage; however, more research would be needed to validate these side effects.

Conclusion

This study aimed to synthesize Paclitaxel conjugated polynitroxylated albumin nanoparticles to be used as a means of increasing the effectiveness of cancer treatment by specifically enhancing the specificity of the drug-carrier for hypoxic tumor cores via nitroxide free radicals. Results showed that the anti-cancer drug was successfully conjugated with modified albumin by means of high pressure homogenization and that nanoparticles of the associated molecules were able to be isolated by centrifugation. Data from the high pressure homogenizer showed that the isolated nanoparticles were of the correct size, 100-200nm, to be used medicinally. Analysis of the electron spin resonance data showed that loading Paclitaxel only slightly altered the nitroxide density initially present in the stock solution of modified albumin used in the experiment. This suggests that the vasodilation and

tumor targeting benefits of the free radicals would most likely be present upon application of the synthesized molecules intravenously; once again, further study would be needed in order to test this. Finally, the absorption data obtained from this study allowed for the quantification of the number of Paclitaxel molecules that were loaded to each modified albumin proteins. When compared to the number of loaded Paclitaxel molecules loaded to unmodified human serum albumin observed in other studies, as well as the Paclitaxel to HSA ratio found in commercially available Paclitaxel, this method allowed for an equal number of Paclitaxel molecules to be loaded to modified PNA. (Paál et al.).

In order to fully quantify the physical characteristics of the synthesized protein complex, more analytical processes will be conducted in the future. One such method will be High Performance Liquid Chromatography (HPLC). The data obtained from running an HPLC analysis will determine the different forms of Paclitaxel that are bound to the modified albumin and the extent to which the cancer drug degraded during preparation. Pending the results of the HPLC analysis, the next step in determining the effectiveness of the synthesized drug carrier protein would be in vitro testing with cancerous cells.

When comparing the absorption peaks in figures 2 and 3 it was observed that there was a decrease in nanoparticle size upon lyophilization. This decrease in size was not expected and further study would be needed in order to determine, and fix, the cause for the change in nanoparticle size. Despite this issue, this study was successful in producing Paclitaxel conjugated polynitroxylated albumin nanoparticles that could prove useful in more effective cancer treatment.

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