Title
Binding Kinetics of Cisplatin with Ion-exchange Resins

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Publication Date
2016

Peer reviewed|Thesis/dissertation

Binding Kinetics of Cisplatin with Ion-exchange Resins

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Biomedical Imaging

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

Acknowledgement

Thank you to my Thesis Committee, Drs. Steven Hetts, David Saloner, Alastair Martin, and Henry VanBrocklin for their guidance. I would also like to thank my lab mates Joshua Fisher, Jay Yu, Bridget Kilbride, Bradford Thorne, Ayushi Gautam, and Caroline Jordan for their insights and assistance. A special thanks goes out to Dr. Mariam Aboiam for her constant encouragement and mentorship.

Binding Kinetics of Cisplatin with Ion-exchange Resins

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Abstract

Objective: Localized chemotherapy can be more effective at treating cancers than traditional chemotherapy methods. Increased dosage leads to increased systemic toxicity, a critical issue that must be addressed. The ChemoFilter - a temporarily deployable, endovascular device - aims to extract chemotherapeutic agents from the bloodstream in order to reduce adverse side effects in other areas of the body. In this study, we report the binding effectiveness of ion-exchange resins with cisplatin, a commonly administered chemotherapeutic.

Materials and Methods: All experiments were conducted *in vitro* using cisplatin in distilled water and phosphate buffered saline. Ion-exchange resins (Dowex 50Wx2, Amberlite FPC22, Tulsion T-66, Amberlite IRC, Purolite S930/950) were tested in solution individually and the total amount of free cisplatin in solution was quantified using ultraviolet-visible spectroscopy and inductively coupled plasma mass spectroscopy.

Results: Quantification of cisplatin using UV-visible methods demonstrated that strong acid cation exchangers perform exceptionally well in saline solutions, removing over 90% of free cisplatin within one minute. The concentration of free cisplatin did not drop when reacted with strong cation exchangers in water. Weak acid cation exchangers and chelating resins also displayed no binding of cisplatin in PBS. Assessing the performance of the strong cation exchange resin, Dowex 50Wx2, using ICP-MS showed that ion exchange filtration was comparable in both water and PBS.

Conclusion: The current effectiveness of localized chemotherapy is limited by its corresponding increased systemic toxicity. The ChemoFilter seeks to mitigate the effects of chemotherapeutics

on non-targeted areas of the body by extracting or inactivating the chemotherapy agents that pass through it. Our benchtop OPDA method of quantifying cisplatin in solution indicated that strong acid cation exchangers were exceptionally well-suited to the task. However, quantification using ICP-MS revealed that our previous UV-visible method of cisplatin quantification was not compatible with ion exchange resin studies and that these resins may not be very useful at fulfilling the ChemoFilter's objective of removing cisplatin from solution.

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Introduction

The rise in popularity of localized chemotherapy can be attributed both to its increased effectiveness and the better quality of life it provides patients. However, the systemic toxicity of chemotherapy treatment is still of great concern and necessitates the need for more efficient localized treatments[1]. A novel intravenous filtering device, the ChemoFilter, seeks to limit this systemic toxicity by removing target drugs from blood before they reach other organs. Although localized intra-arterial treatments reduce the amount of chemotherapeutic that can cause systemic toxicity, there is still a high bypass rate and large doses of chemotherapeutic can disperse and accumulate in non-target organs. For instance, the cardiac toxicity associated with hepatocellular carcinoma, is the result of up to 60% of doxorubicin bypassing the tumor and reaching the heart[2,3]. In its current form, the ChemoFilter will be temporarily deployed endovascularly through image guidance and removed upon completion of the treatment session. The proximity of the device to the target organ and its location in the exiting vasculature offers the potential to significantly reduce toxicity of treatment of different types of cancers (Figure 1). The ChemoFilter aims to reduce the severity of incurred side effects through rapid removal of chemotherapeutics and improve treatment quality by increasing the allowed dosage of the agent administered.

Cisplatin (CDDP), along with doxorubicin and paclitaxel, is one of the most widely used chemotherapeutic agents due to its efficacy in treating a wide array of cancers[4,5] and is often administered intravenously as an infusion in saline and other agents. Cisplatin is a very effective chemotherapeutic, but is dose-limited due to its neurotoxicity, ototoxicity, emetogenesis, and nephrotoxicity[6]. In particular, the severe adverse renal effects necessitate efficient removal of CDDP from blood if higher doses are to be administered. However, there is no clear mechanism by which CDDP and other platinum complexes are filtered out of solution. Various methods of

agent removal have been proposed and it has been shown that doxorubicin can be extracted from blood using both ion-exchange resins and DNA[7,8].

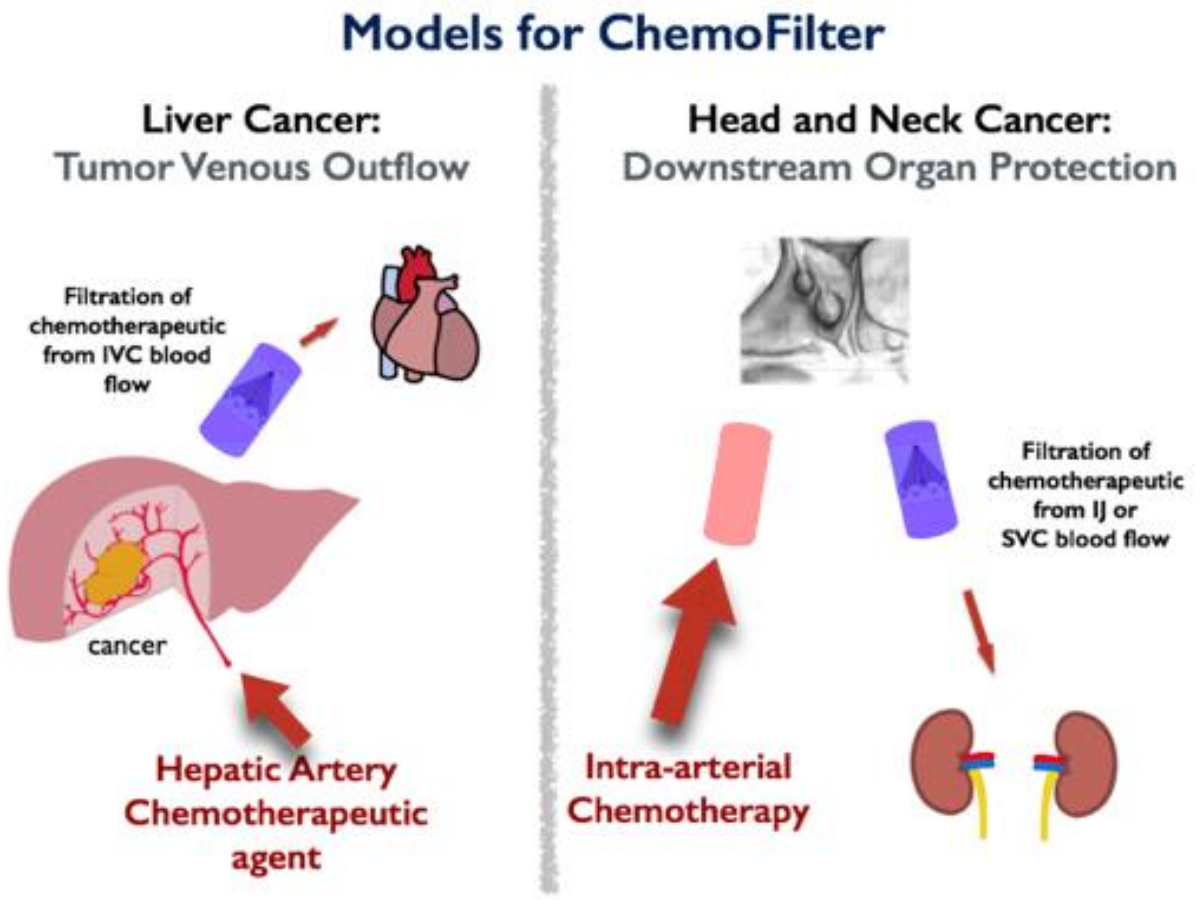


Figure 1: Preliminary device design and potential locations for ChemoFilter placement. The Chemofilter's structure and filtration mechanism may be specifically modified to target select cancer treatments.

The chemotherapeutic benefits of cisplatin can be attributed to its role in disrupting cell signaling pathways, causing apoptosis. The chlorine ligands act as leaving groups, substituted by a covalent bond to the nucleophilic N^7 position of purine bases. Having two available leaving groups, cisplatin can then bind with pairs of nearby purines to form intrastrand crosslinks. In particular, the guanine-guanine adduct is thought to be responsible for cisplatin's anticancer properties. *In vivo* preference for thiol groups and other sulfur containing compounds have also been documented and play a role in cisplatin resistance over time[9-11].

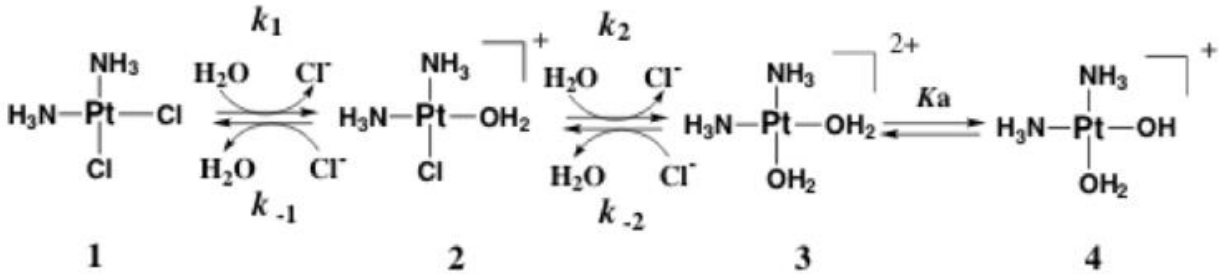


Figure 2: Cisplatin is administered in the chlorinated, chemotherapeutically inactive form. In the body, it undergoes a series of hydration steps where the chloride ions are replaced with water. The final positively charged compound then crosslinks to DNA, interrupting cell signaling processes.

Clinically, cisplatin is administered in its neutrally charged, chlorinated form. In this state, cisplatin is considered chemotherapeutically inactive. CDDP becomes hydrated or "aquated" in the body (Figure 2), with water replacing one or both chloride ions and imparting a positive charge[12]. The ChemoFilter plans to target this charged cisplatin using ion exchange resins, which are essentially polymers with charged groups covalently attached. This study seeks to investigate the binding properties and kinetics of CDDP to ion exchange resins in order to develop an efficient *in vivo* chemotherapeutic filtering device.

Materials and Methods

Materials

Reagents were obtained from commercial sources and used as supplied. Cisplatin (1 mg/ml, 0.33 mM working solution) and Dowex 50Wx2 were purchased from Sigma Aldrich (St. Louis, MO); Amberlite FPC22 from Alfa Aesar (Ward Hill, MA); Tulsion T-66 from Thermax Limited (India); O-phenylenediamine (OPDA) was purchased from MP Biomedicals, LLC (Solon, OH); phosphate-buffered saline (PBS) was purchased from Thermo Fisher (Waltham, MA); distilled water was retrieved from a Millipore Milli-Q system. All solvents were reagent grade.

Figure 3: Dowex 50Wx2 beads.

Binding experiments were performed *in vitro* with ion exchange resins in distilled water and PBS. All *in vitro* experiments were performed at room temperature.

Initial proof of concept

Cisplatin interaction with Dowex was first investigated in a chemically neutral environment, distilled water. The same experiment was then conducted using PBS instead of water to better simulate physiological conditions. A 100 mg/m2 clinical dose of cisplatin was diluted, assuming an average human body surface area of 1.7 m^2, with

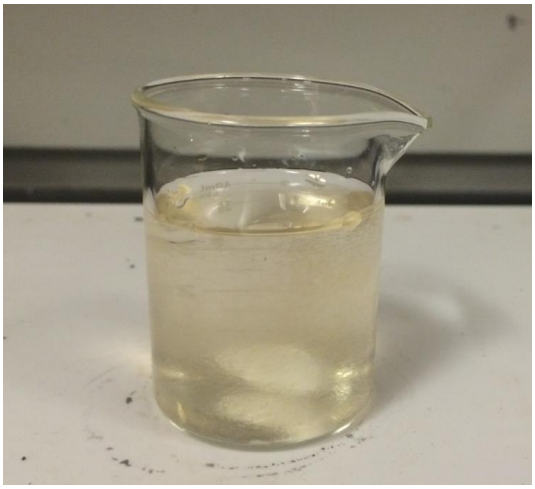


Figure 4: Experimental setup with 2.0 g Dowex in 50 mL PBS. Dowex imparts a slight orange coloration to the solution.

both solvents to achieve 0.05 mg/mL concentrations[13]. Two grams of Dowex were added to both solutions and a magnetic stir bar was used to roughly simulate fluid flow. 300 uL samples were taken at times 0, 1, 3, 5, 7, 10, 12, and 15 minutes after its introduction. Quantification of free cisplatin was achieved through UV visible spectroscopy of the extracted samples.

Varying Dowex Concentrations

0.05 mg/mL cisplatin in PBS solution was reacted with the following amounts of Dowex: 2.0 g, 1.5 g, 1.0 g, 0.75 g, 0.5 g, 0.3 g, and 0.19 g. Samples were taken at the same time points as before and quantified using OPDA. The procedure was performed twice more after identifying the ideal amount of Dowex so as to establish the degree of reproducibility in the measure.

Additional resin testing

Other strong acid cation exchangers, including Amberlite FPC22 and Tulsion T-66, were tested to verify their effectiveness at removing cisplatin from PBS. Weak acid cation exchangers and chelating resins were assessed as well. All experiments on resins other than Dowex were conducted using the same protocol as our initial proof of concept experiment with PBS as the solvent.

Quantification using o-phenylenediamine (OPDA)

In order to determine the concentration of free cisplatin in solution it was complexed with OPDA. 600 uL of 4.5 mM OPDA in PBS was added to each sample and boiled at 95-100 ºC for 10 minutes to allow for complexing. Samples were placed in an ice bath to cool after which 2.1 mL of dimethylformamide (DMF) was added to each in order to precipitate any remaining free cisplatin and halt the reaction with OPDA. The resulting compound forms a green color and exhibits a characteristic absorbance peak at 706 nm[14]. Spectroscopy was subsequently performed using a Digilab Hitachi U-2810 spectrophotometer and UV Vis software. A standard curve for cisplatin absorbance was created by plotting measured absorbance values against their specific CDDP concentrations (0-0.07 mg/mL) and deriving a first-order linear trendline. Experimental absorbance values were then converted to mg/mL units by fitting to the generated trendline.

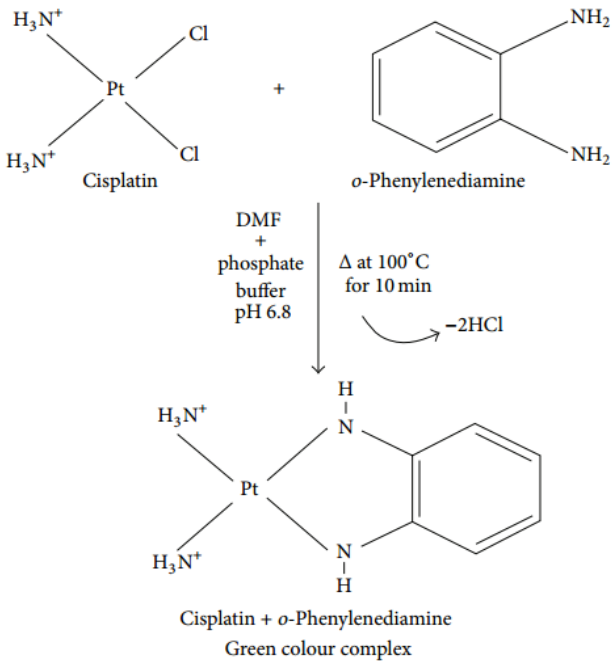


Figure 5: Complexing reaction of CDDP and ODPA to spectroscopically quantify CDDP concentration in solution[14].

Quantification using inductively coupled plasma mass spectroscopy (ICP-MS)

Atomic absorption spectroscopy and ICP-MS are considered the gold standards for quantifying metals, including platinum, in solution. A plasma source powered by electricity generated from electromagnetic induction is used to ionize the sample solution. Ionized particles are then quantified with the attached mass spectrometer. Our ICP-MS data was acquired on a Perkin Elmer

Optima 7000 DV system, courtesy of the Balsara Lab at UC Berkeley, at 265 nm. Calibration was performed every three hours using specifically prepared concentration standards (0, 1, 3, 5, 10, 25, 50, 100 and 150 ppm) made from pure cisplatin and PBS stock solutions. Parts per million units were then correlated to mg/mL in the same linear fashion as with UV spectroscopy.

Results

Initial proof of concept: Water vs PBS

To observe the effectiveness of ion exchange resin, Dowex was administered in a chemically neutral solvent, distilled water, and then in PBS, which more closely resembles *in vivo* conditions. Reacting Dowex with cisplatin in water showed a slight increase in free cisplatin during the first minute after which the concentration remained approximately the same. While this was both an unintended and undesirable result, di H_20 is a poor model for blood. Fortunately, in PBS, where the osmolarity and ion concentrations equal that of blood, Dowex demonstrated excellent kinetics, binding >90% of cisplatin within one minute (Figure 6).

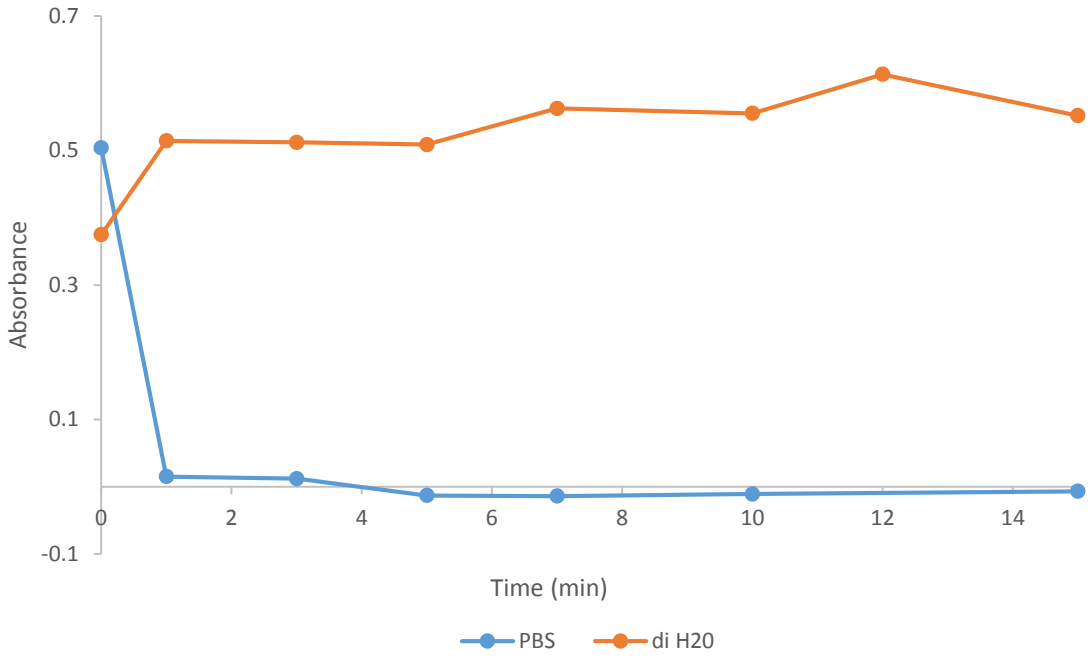
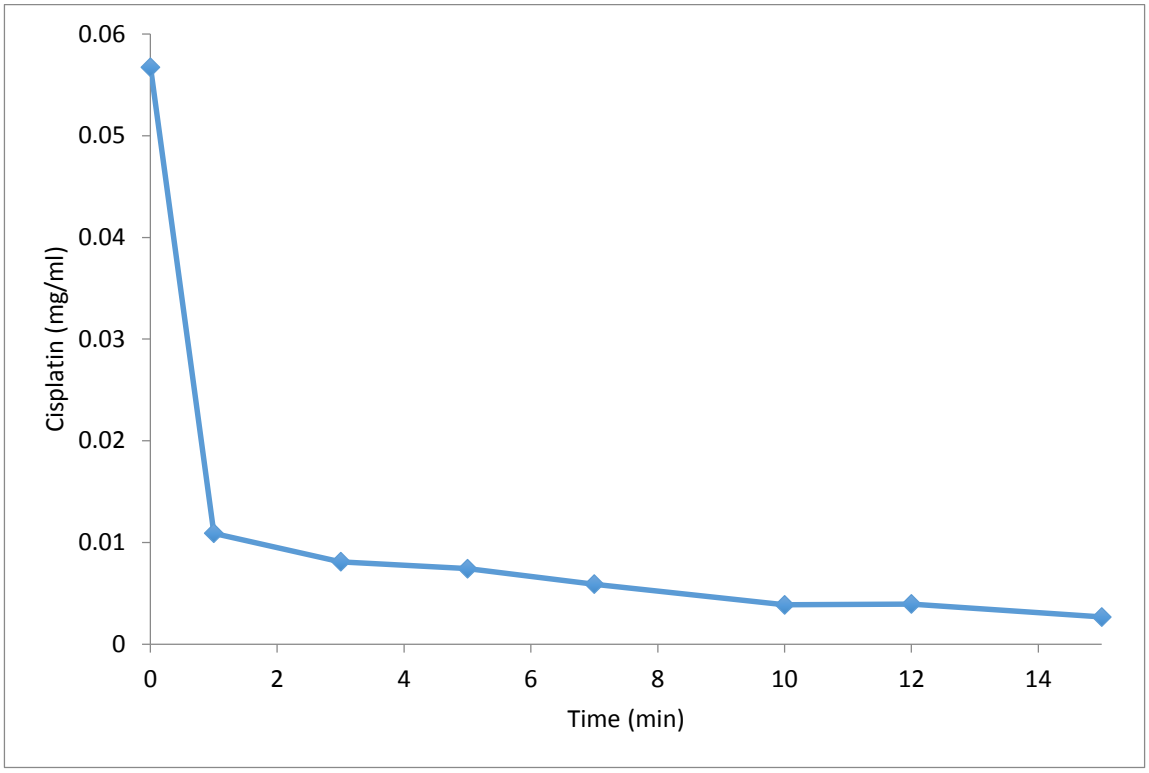


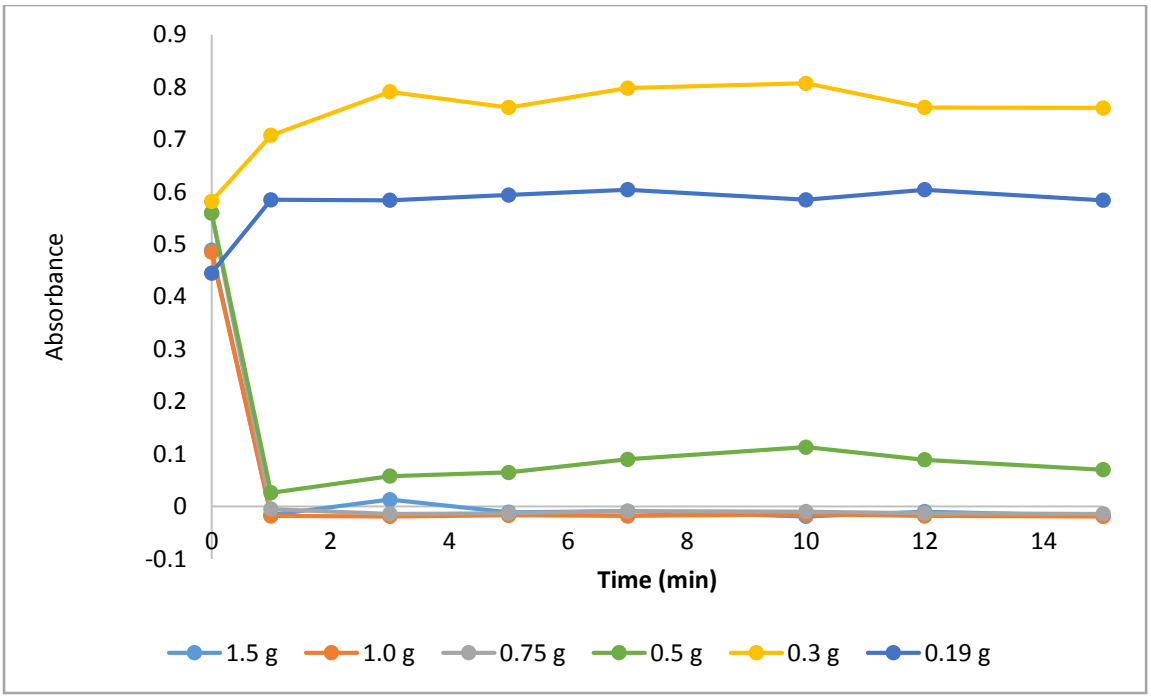
Figure 6: Reaction profiles for 2.0g of Dowex with cisplatin in water (di H_20) and PBS. An overall slight increase in absorbance was observed in water while spectroscopic evidence of cisplatin was not present after one minute in PBS.

Optimal amount of Dowex

Testing different amounts (0.19-2.0 g) of Dowex revealed that 0.5 g was capable of removing over 80% of free cisplatin within one minute (Figure 7 a). Amounts greater than 0.5 g shared similar reaction profiles and demonstrated 100% clearance. Smaller amounts resulted in reaction profiles similar to Dowex in water, initially exhibiting a slight increase in cisplatin concentration and then remaining relatively constant (Figure 7 b).

(a)



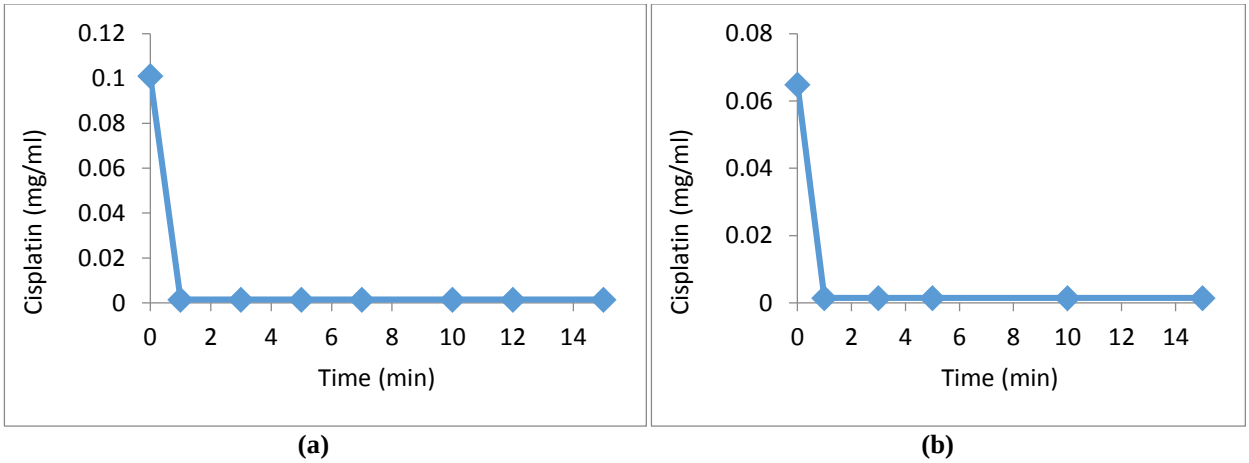


(b)

Figure 7: (a) Average cisplatin concentration of a triplicate of 0.5g Dowex in PBS illustrates the steep drop that occurs in the first minute. (b) Reaction profiles for varying amounts of Dowex in PBS. Activity threshold lies clearly around 0.5g, above which the reaction proceeds quite rapidly.

Additional strong cation exchangers

Given the strong binding kinetics displayed by Dowex, we sought to compare them to other available strong cation exchangers. Both Amberlite FPC22 and Tulsion T-66 exhibited excellent binding, with almost 100% capture in the first minute (Figure 8 a,b). These strong cation resins share the same sulfonic acid group as Dowex and have similar copolymer structures as well.

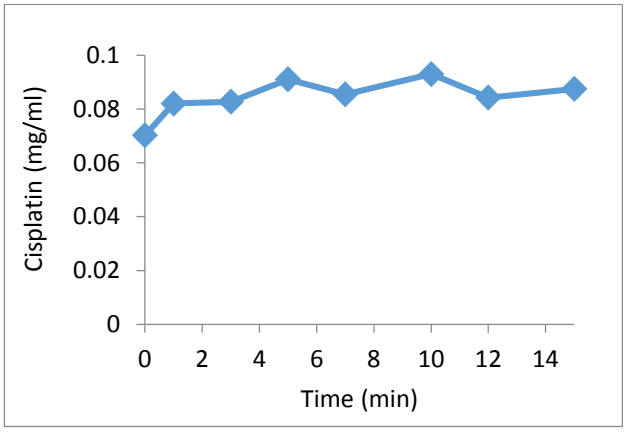


(a) **(b)**

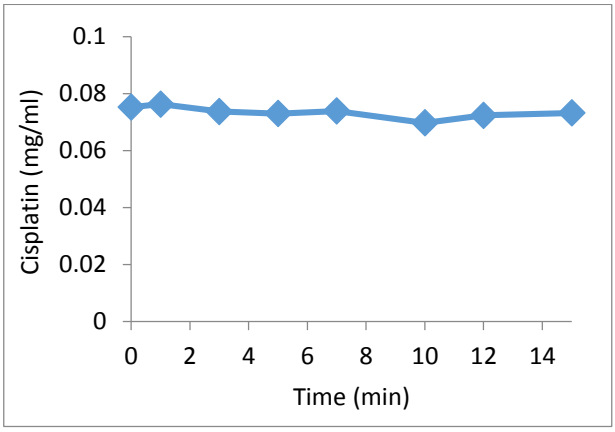
Figure 8: (a) 2.0g Amberlite FPC22 in PBS and (b) 2.0g Tulsion T-66 in PBS. Almost all cisplatin was filtered out of solution within one minute.

Weak cation exchanger and chelating resins

The weak cation exchanger, Amberlite IRC86, demonstrated no noticeable binding though there was a minor increase in cisplatin concentration during the first minute (Figure 9a). Due to difficulties procuring additional weak cation exchangers, chelating resins, Purolite S930 (Figure 9b) and S950 (Figure 9c), were explored. These resins utilize iminodiacetic acid and aminophosphonic acid respectively to covalently bind metal cations, with the central nitrogen atom displaying strong affinities for heavy metals. However, as the platinum atom of active CDDP is sterically hindered by its four ligands, there was no observed drop in cisplatin concentrations.



(a)



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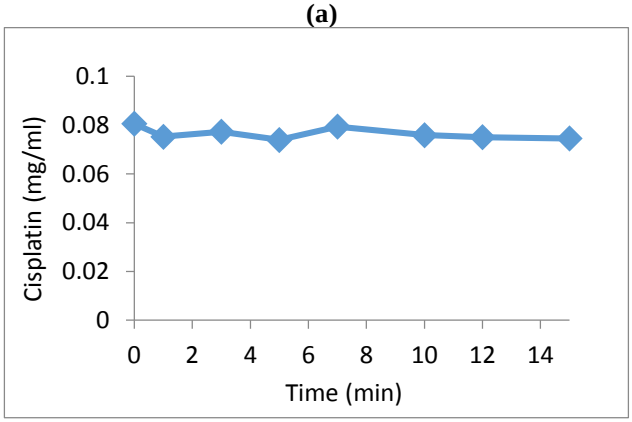


(c)

Figure 9: (a) 0.5g of the weak cation exchange resin Amberlite IRC86 in PBS. A very slight increase in CDDP was observed in the first minute followed by slight fluctuations for the duration of the trial. Chelating resins: (b) 0.5g Purolite S930 in PBS, and (c) 2.0g Purolite S950 in PBS. No change in cisplatin concentration was observed. The reaction profile showed very little variation over time.

Different ion-exchange resins results

Comparing results between different resin categories confirmed that strong acid cation exchange resins display the best clearance, with all three sharing almost identical reaction profiles. Both chelating resins shared similar profiles as well, exhibiting essentially no change in cisplatin absorbance. Notably, the weak cation exchanger showed an increase in CDDP, ending with a higher absorbance than either chelator and showing a trend opposite to the strong cation exchangers.

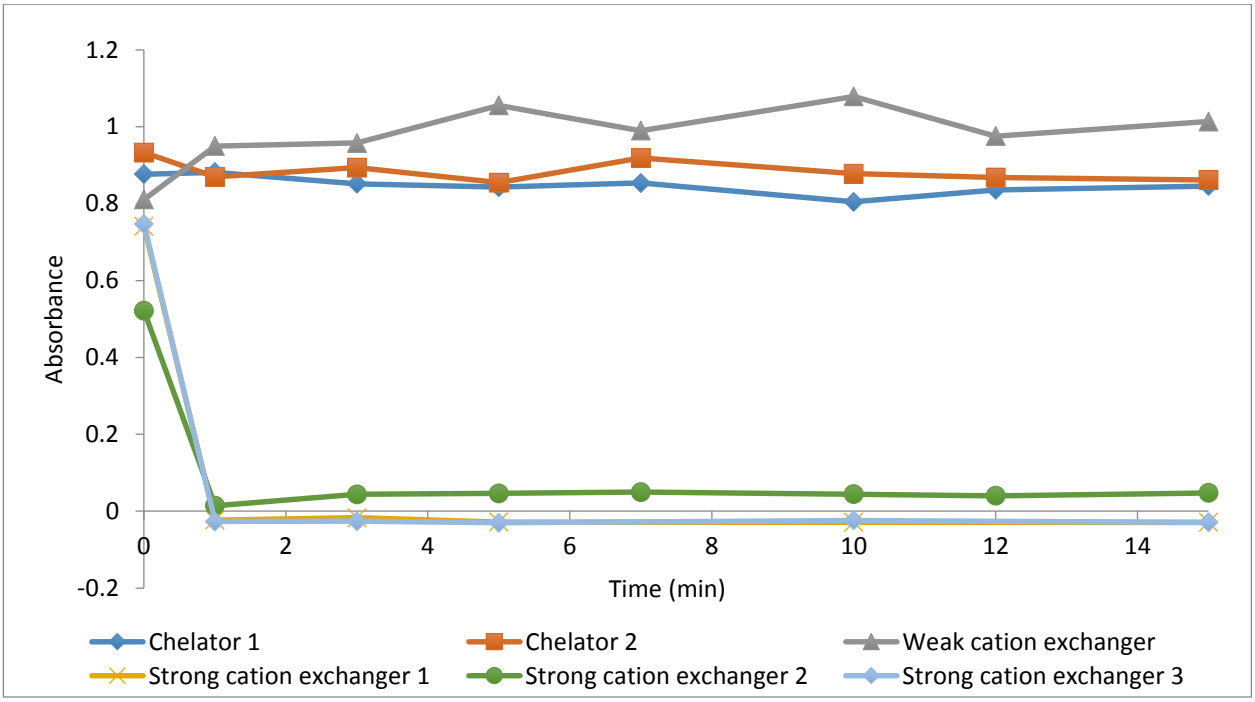
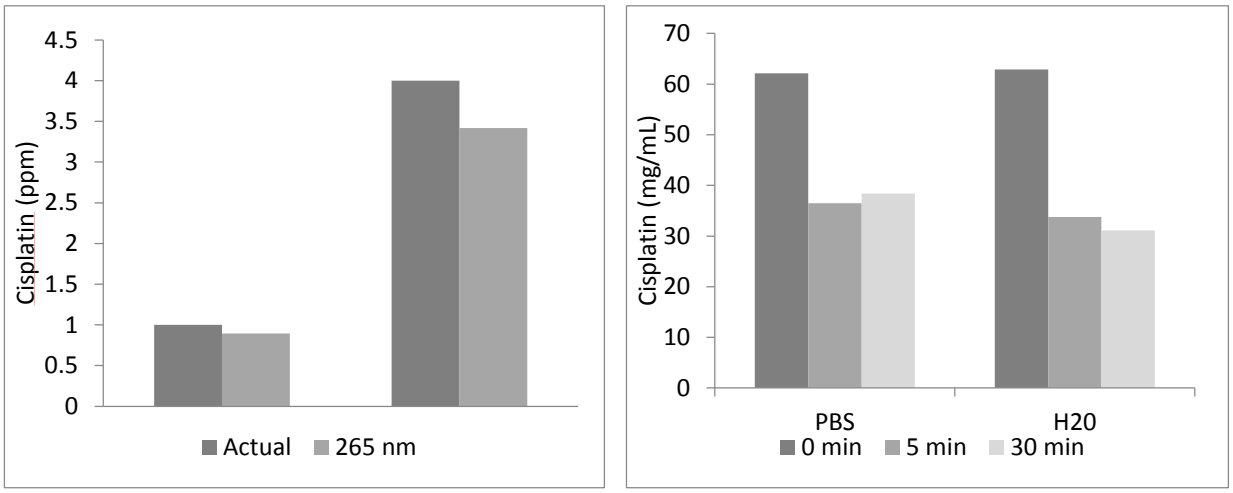


Figure 10: A comparison of the various types of resins studied. The chelating resins and weak cation exchanger demonstrate moderately stable absorbance values over the testing period. Conversely, the strong cation exchangers exhibit steep drops in absorbance within the first minute. Considering the linear relationship between cisplatin absorbance and concentration, strong cation exchangers clearly demonstrate the best filtration performance of all tested resin types.

Quantification with ICP-MS

Standard curves were generated by diluting stock solutions of cisplatin and PBS to precisely known concentrations and verified with ICP-MS. Accurate concentration readings confirmed that ICP-MS is effective at quantifying free cisplatin in solution. A repeat of our proof of concept

experiment was performed, taking samples at 0, 5, and 30 minutes. Cisplatin concentrations in both water and PBS stabilized within five minutes and in both solutions the total CDDP concentration dropped by 40-50%.



(a) **(b)**

Figure 11: (a) Standard curve measurements using ICP-MS confirming accurate quantification of cisplatin concentration (b) Repeating our initial proof of concept experiment showed relatively constant cisplatin concentrations after 5 min in water and PBS. The overall drop in concentration did not match the findings which used OPDA for quantification.

Discussion

Dowex 50Wx2 was chosen as the initial resin for testing due to prior testing[15] demonstrating successful clearance of doxorubicin from PBS and serum. The initial proof of concept experiment also demonstrated excellent binding of cisplatin to Dowex in PBS, but no binding in water. These results were somewhat surprising as we had expected cisplatin to exist predominantly in its hydrated form in water and therefore should display evidence of CDDP-resin interactions. The high chlorine concentration of PBS was thought to minimize ligand substitution and would therefore not interact with ion exchange resins. However, given water's poor resemblance to physiological conditions, and preliminary data that demonstrated apparently excellent binding activity with PBS, we decided to continue investigating ion exchange properties using only PBS as the solvent.

Testing variable amounts of Dowex indicated that its interaction with cisplatin was dose dependent, displaying approximately 80-100% binding for amounts greater than 0.5 g and poor performance for amounts less than 0.5 g. Excellent binding kinetics were also observed for the other strong cation resins. The weak cation exchanger exhibited a reaction profile similar to that of Dowex in water, with the overall CDDP concentration rising during the first minute. This was contrary to our assumption that the weak cation exchangers would have a noticeable, but minor, effect on free cisplatin concentration. Additional weak cation exchangers were not readily available. Instead, chelating resins, applications of which include removal of heavy metals from solution, were investigated to determine their effectiveness with cisplatin. We found that these resins displayed the least amount of activity, with CDDP concentrations remaining relatively constant over the entire observed period. Comparing all of the resins seemed to indicate that the strong cation exchangers were perfectly applicable to the ChemoFilter, yet we remained doubtful

because the observed sub-minute clearance rates were considerably better than in our doxorubicin studies[15].

Furthermore, difficulties encountered when replicating the data continued to raise suspicions that our previous results may not be accurate. While we were able to generate triplicates for many of the resins, the absorbance readings from our spectrophotometer would fluctuate significantly on a weekly basis regardless of calibration. Visual confirmation of the OPDA method was also inconsistent. The OPDA-CDDP complex should turn green and exhibit an absorbance peak at 706 nm. However, the spectrophotometric readings of our processed, green colored solutions often showed no drop, or random increases, in free CDDP and clear or yellow colored samples sometimes demonstrated the ideal reaction kinetics. This variation was troubling and we sought to quantify our cisplatin solutions using more trustworthy means.

In collaboration with the Balsara Lab at the University of California, Berkeley, we established standard curves for cisplatin at 265 nm and were able to confirm accurate measurements using ICP-MS. Repeating our initial proof of concept experiment now showed very different results depending on the quantification method. Rather than the sub-minute inactivation in PBS we observed a 38.2% decrease in cisplatin over five minutes. In water, free CDDP decreased by 50.5%. Recent attempts at reproducing these drops in concentration using ICP-MS have not been successful but the final cisplatin concentrations in PBS and water consistently remain similar.

These ICP-MS findings suggest that our benchtop OPDA method of quantification is ineffective. The dissociate properties of strong cation exchangers acidify the solution, altering the chemical composition and potentially disturbing the complexing reaction between OPDA and CDDP. However, using the OPDA method showed the weak cation exchange resin actually performed worse than the chelators. This discrepancy in the performance of the strong and weak

cation exchangers suggests that our addition of these resins interferes with the OPDA-CDDP reaction by altering the solution chemistry, thus invalidating our use of this quantification method.

We shall attempt to confirm the OPDA method by using pure cisplatin, rather than clinical grade cisplatin, to generate a standard curve to rule out any effects that the carrier salt solution may have on the complexing process. However, moving forward, all quantification will be accomplished using ICP-MS. Previous experiments will also be performed again and quantified using ICP-MS to verify the effectiveness of ion exchange for ChemoFilter. Development of this iteration of the ChemoFilter has proved challenging due to lack of published literature on CDDP's binding mechanism. Further studies on cisplatin's *in vivo* affinities may reveal the ideal active groups that can be covalently attached to the device's physical structure.

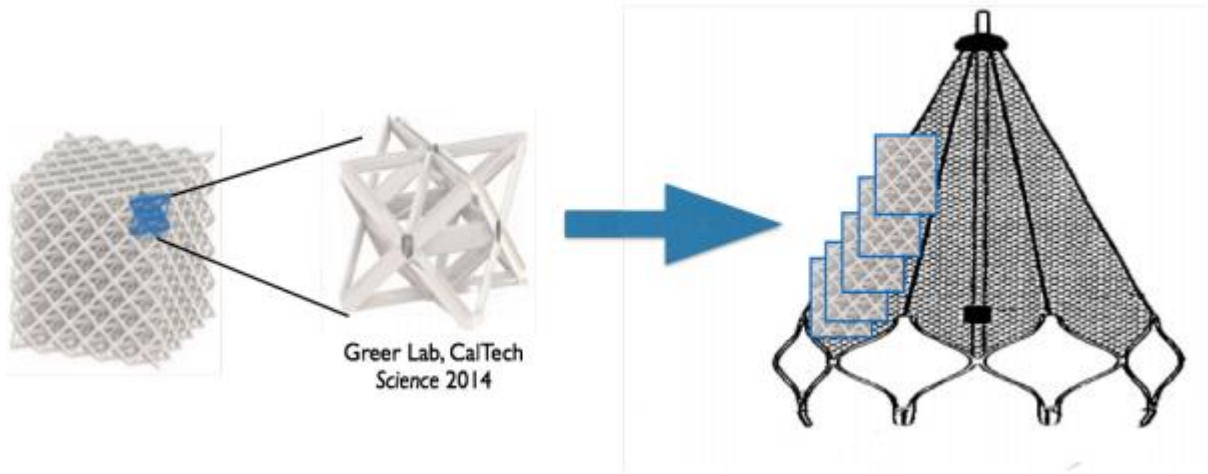


Figure 12: Prototype nanotruss and application on the ChemoFilter. Truss geometry can be configured to orient covalently attached groups in specific directions or allow passage of different chemotherapeutic drugs.

Current versions propose use of a synthetic nanotruss (Figure 12) onto which we can attach specific functional groups for ion exchange or specific oligonucleotides. Other potential designs include permeable membranes[16] with binding sites attached and oriented specific to the size of the target chemotherapeutic. Theoretically, both designs should increase the surface area available for

drug capture and the final design must also take into account specific venous flow rates and the strength of the selected binding mechanism. As of now, the high affinity of doxorubicin for DNA and cisplatin's specific targeting of the N^7 position on purine rings point to DNA as the most effective ligand for a multidrug ChemoFilter.

Conclusion

Localized chemotherapy has the potential to overtake conventional chemotherapy if the caveat of increased systemic toxicity is addressed. In particular, treatment of cancers such as liver cancer which do not respond to current chemotherapy options and cannot be surgically resected will become significantly more effective. The ChemoFilter seeks to limit the amount of chemotherapeutic agents that reach other areas of the body. This study investigated the potential for ion exchange resins to filter cisplatin from PBS, a simple blood analog. We found that, when using OPDA to quantify cisplatin concentration, strong acid cation exchangers exhibited exceptional binding kinetics, removing over 90% of free cisplatin within one minute. Weak acid cation exchangers and chelating resins displayed no decrease in cisplatin under this method as well. However, quantifying cisplatin using ICP-MS demonstrated that our previous benchtop OPDA method was not reliable. Resin experiments will be repeated to determine the validity of our previous measurements as well as the true effectiveness of ion exchange. Nonetheless, moving forward, DNA seems to offer higher potential for multidrug capture

References

1. Porrata LF, Adjei AA. “The pharmacologic basis of high dose chemotherapy with haematopoietic stem cell support for solid tumours.” Br J Cancer 2001. 85:484-489.
2. Hwu WJ, Salem RR, Pollak J, et al. “A Clinical-pharmalogical evaluation of percutaneous isolated hepatic infusion of doxorubicin in patients with unresectable liver tumors.” Oncology Research 1999. 11:529-537.
3. Curley SA, Newman RA, Dougherty TB, et al. “Complete hepatic venous isolation and extracorporeal chemofiltration as treatment for human hepatocellular carcinoma: a phase I study.” Ann Surg Oncol 1994. 1:389-399.
4. Kelland L. “The resurgence of platinum-based cancer chemotherapy.” Nat Rev Cancer 2007. 7:573–584.
5. Loehrer PJ, Einhorn LH (May 1984). "Drugs five years later. Cisplatin." Annals of Internal Medicine 100 (5): 704–13.
6. Madias NE, Harrington, JT. “Platinum nephrotoxicity.” Am J Med 1978. 65:307–314.
7. Satoshi Tanida, Tsutomu Mizoshita, Keiji Ozeki, et al., “Mechanisms of Cisplatin-Induced Apoptosis and of Cisplatin Sensitivity: Potential of BIN1 to Act as a Potent Predictor of Cisplatin Sensitivity in Gastric Cancer Treatment.” International Journal of Surgical Oncology, vol. 2012, Article ID 862879, 8 pages, 2012.
8. Yan X, Gemeinhart RA. “Cisplatin delivery from poly(acrylic acid-co-methylmethacrylate) microparticles.” Journal of Controlled Release 2005. 106:198-208.
9. Dabrowiak JC, Goodisman J, Souid A. “Kinetic Study of the Reaction of Cisplatin with Thiols.” Drug Metabolism & Disposition 2002. 30(12): 1378-1384.

10. Hall MD, Telma KA, Chang K-E, et al. “Say No to DMSO: Dimethylsulfoxide Inactivates Cisplatin, Carboplatin and Other Platinum Complexes.” Cancer research 2014. 74(14):3913-3922.
11. Surnar B, Sharma K, Jayakannan M. “Core–shell polymer nanoparticles for prevention of GSH drug detoxification and cisplatin delivery to breast cancer cells.” Nanoscale 2015. 7: 17964-17979
12. Wilson J, Lippard SJ. “Synthetic Methods for the Preparation of Platinum Anticancer Complexes.” Chem Rev 2014. 114(8): 4470–4495.
13. Sparreboom A, Verweij J. "Paclitaxel Pharmacokinetics, Threshold Models, and Dosing Strategies." Journal of Clinical Oncology 2003. 21 (14): 2803–4.
14. Basotra M, Singh SK, Gulati M. “Development and Validation of a Simple and Sensitive Spectrometric Method for Estimation of Cisplatin Hydrochloride in Tablet Dosage Forms: Application to Dissolution Studies.” ISRN Analytical Chemistry, vol. 2013, Article ID 936254, 8 pages, 2013.
15. Yu, J. “Development of filter device to limit systemic toxicity from doxorubicin chemotherapy: DNA ChemoFilter.” Master’s thesis, University of California, San Francisco, 2015. ProQuest (AAT 1600669).
16. Chen CX, Oh HJ, Yu J, Yang J, Petzetakis N, Patel AS, Hetts SW, Balsara NP. “Block Copolymer Membranes for Efficient Capture of a Chemptherapy Drug.” ACS Macro Letters 2016. 5:936-941.

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