Issues with metals in nucleotide based pharmaceutical production

Technical Insight

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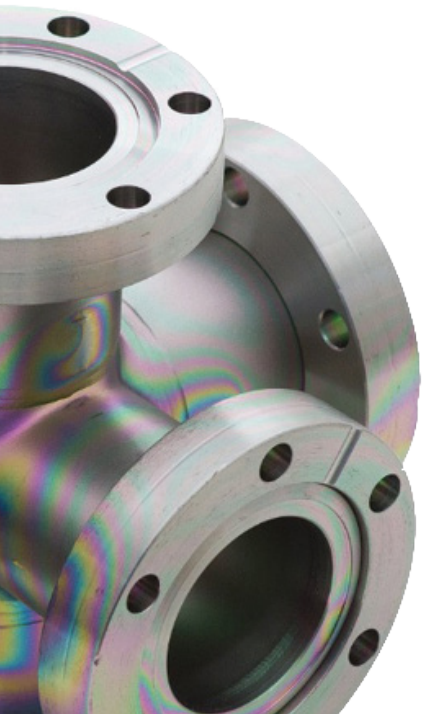

Synopsis

Nucleotide-based pharmaceuticals are a relatively new, but rapidly growing therapeutic modality. Metals and metal ions can interact with nucleotide-based therapeutics in a variety of negative ways. Eliminating these negative interactions can be accomplished by coating the stainless steel commonly used in both production and purification equipment for these molecules. This TI will discuss a brief history and background of nucleotide therapeutics, how metals can negatively impact their production, and how Dursan can help alleviate these issues.

Background

Nucleotide-based therapeutics are a relatively new, but rapidly growing modality of treatment. They differ from traditional small molecule treatments due to their ability to work in and with the cells of the body to alter the production and expression of proteins. There are a variety of different nucleotide-based therapeutics, but they are all based on nucleotides linked via a phosphate backbone, just like our RNA and DNA. Types of these therapeutics include but are not limited to oligonucleotides (antisense oligonucleotides and small interfering RNA) and messenger RNA (mRNA). Figure 1 shows the rise in publications since 1985 on these three different modalities (note that not all of these publications discuss therapeutics). The emergency use of mRNA-based vaccines was critical in fighting the COVID-19 pandemic. Additionally, there are 15 oligonucleotide treatments that are FDA approved, and many more currently in clinical trials.

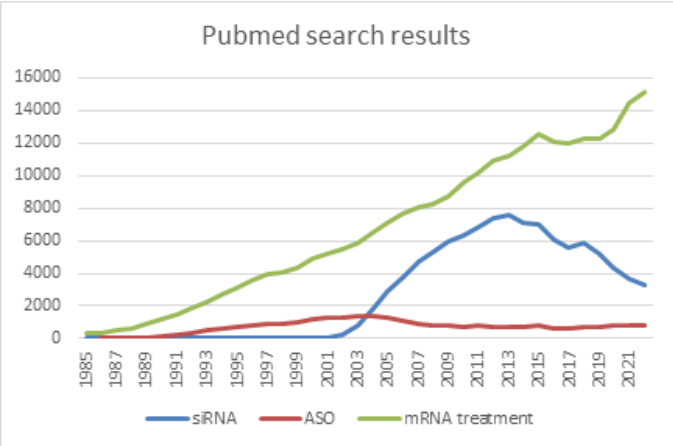

Figure 1: Published papers that mention small interfering RNA, antisense oligonucleotide, or mRNA treatment from 1985 to 2022.

The manufacturing process of these treatments requires analysis of impurities and purification of the end product. Liquid chromatography plays a key role in nearly all aspects of this process. The pumps, tubing, valves, and columns used in chromatography are typically made of stainless steel which is an alloy of iron, chromium, nickel, and a variety of other minor metallic elements depending on the grade of steel. This can be problematic for two reasons.

First, nucleotides are well known to chelate to metal surfaces as well as metal ions in solution.[1,2]
Aptamer (another nucleotide-based material) biosensors take advantage of this chelation when performing environmental monitoring for potentially toxic heavy metals.[3] With the potential to chelate metal surfaces, nucleotides are prone to recovery issues. This can be a problem when performing impurity analysis as many impurities are in trace quantities and they may go unnoticed if too many stick to the metal flow path. Additionally, the formation of metal adducts can cause detection issues, especially when performing mass spectroscopy.[4]
Second, metal ions are oxidative in nature. The phosphate linker between nucleotides can be phosphorothioates (PS) or phosphates (PO). It is not within the scope of this TI to go into detail about PS and PO oligonucleotides but these states are important to the therapeutic efficacy of an oligonucleotide and oxidative stress can cause a PS to PO transition. The issue with metal ions in solution is they can chemically degrade a target nucleotide-based compound at multiple stages in the manufacturing process.

YMC Europe put together a thorough review of the analysis of and purification of oligonucleotides via HPLC. It discusses ion-pairing reversed phase chromatography as a method to perform these separations. Analytes that are highly polar, such as oligonucleotides due to the phosphates along the backbone of the molecule, typically are not well retained on a reverse phase HPLC column. To solve this issue, a small amount of an ion pairing reagent is added to the mobile phase. These ion pairing reagents have a charged portion that binds to the oligonucleotide and an alkyl portion that can interact with the reverse phase silica and cause separations.

There is very little information in the literature that describes the impact of these ion pairing reagents on metal surfaces such as stainless steel. In this TI we quantify the metal ion leaching effect of ion pairing solvents systems that are commonly used for UV and mass spec detection. A typical UV solvent system consists of water and acetonitrile with triethylammonium acetate (TEAA) as the ion pairing reagent. Mass spec analysis utilizes triethylamine (TEA) and hexafluoroisopropanol (HFIP) in water and methanol. The impact metal ion leaching from these solvent systems has on oligonucleotide recovery will also be discussed.

Data and Discussion

Sintered metal filtration media were utilized as the stainless steel coupons due to their high surface area. Each sample was a round 2 µm nominal pore sized frit that measures 1/4" in diameter and are 1/8" thick. Samples were cleaned with a citric acid-based detergent to ensure no impurities were present on the surface and that the material is well passivated prior to testing. Half of the samples were then coated with Dursan prior to being introduced to the solvent systems. These solvent systems are described in Table 1. Each sample was also soaked in 100% A and 100% B solvent systems without the TEAA, TEA, and HFIP to isolate the impact of the additive itself. In most HPLC analyses, a mixture of aqueous and organic solvents is used, so A and B solvents were mixed in 20% increments and tested. Additionally, each solvent system in this study was exposed to the same conditions without a metal sample for control purposes.

	UV Mobile Phase	Mass Spec Mobile Phase
Mobile Phase A	100 mM TEAA in water	15 mM TEA, 400 mM HFIP in water
Mobile Phase B	100 mM TEAA in acetonitrile	Mobile phase A + Methanol (50:50 v/v)
Temperature	60°C	60°C
Exposure time	1 week	1 week

Table 1: Solvent systems used for metal ion leaching studies.

Samples were soaked in the appropriate solvent systems in triplicates. There was visually a difference in some samples after this step as seen in Figure 2. The visual indication correlated well with samples that leached high amounts of iron into the solution tested.

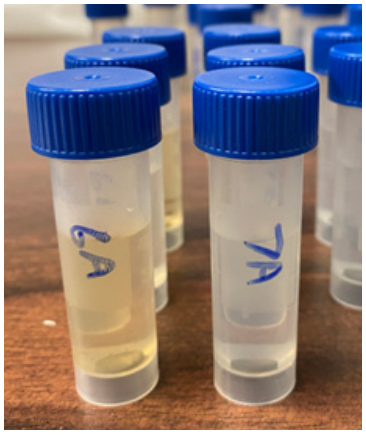

Figure 2: Tube on the left (labeled 6A) is the UV Mobile phase 0% A with an uncoated metal coupon. It shows a yellow color which correlated to high levels of iron. The right is the same mobile phase with the Dursan coating. The clear color shows minimal metal introduction to the solution.

After the soak, the metal sample was removed and the solvent evaporated in air. All residual solids after evaporation were redissolved with nitric acid and analyzed via ICP-MS analysis on a Thermo Scientific iCAP RQ system. The results were corrected by subtracting metal concentrations from the control groups that did not see a metal sample. The results can be seen in Figure 3 for the UV mobile phase and Figure 4 for the mass spec mobile phase.

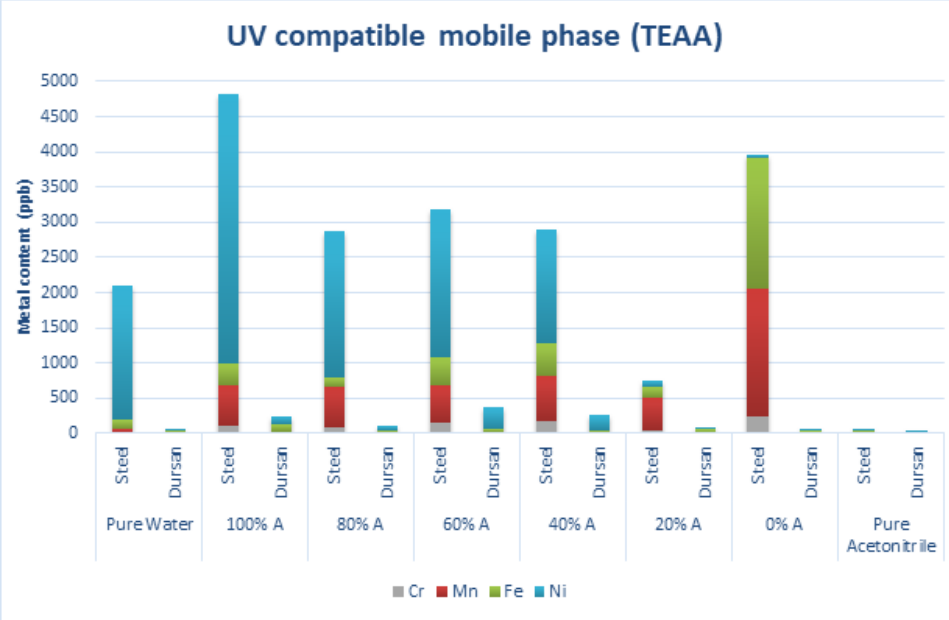

Figure 3: Metal ion leaching from a UV compatible mobile phase for oligonucleotide analysis.

TEAA has a negative impact to the metal ion leaching (see Pure water and pure acetonitrile that has no TEAA compared to 100% A and 0% A). The manganese contribution in water is surprising considering it only accounts for 2% of stainless steel's bulk composition. There is also a dramatic difference between the TEAA in water and the TEAA in acetonitrile. In water it selectively attacks the nickel. This may be due to the water itself rather than the TEAA, but it appears to be exacerbated by the presence of the TEAA. Unlike the water, the acetonitrile does not have a significant impact to the stainless steel on its own. Water has shown to be more aggressive than acetonitrile in previous studies done at room temperature. It is only when the TEAA is added that significant leaching occurs. The 0% A mixture shows a selective attack on iron and manganese when compared to all other samples in this round of testing. There is no obvious pattern in the ratios between aqueous and organic phases other than all mixtures having less overall metal ion leaching than either of the individual components. Regardless of the aqueous or organic content of the solvent, Dursan performed well across the board blocking metals from leaching into the solution.

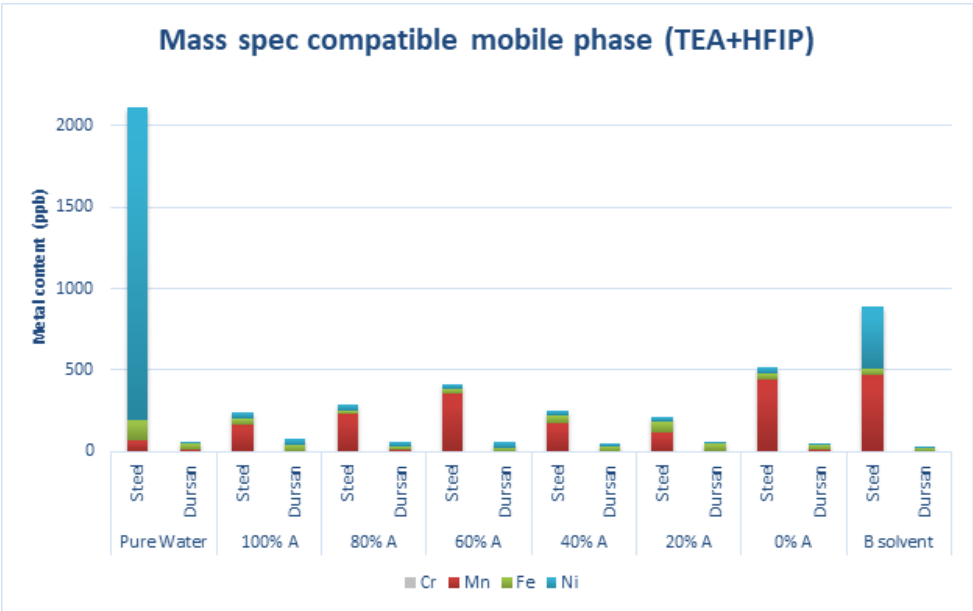

Figure 4: Metal ion leaching from a mass spec compatible mobile phase for oligonucleotide analysis. B solvent is a 50:50 mixture of pure water and pure methanol without the addition of TEAA and HFIP.

Unlike TEAA, the mass spec solvent system saw a decrease in the amount of metal ions leached when compared to pure water and the B solvent (50:50 mixture of water and methanol) without the addition of TEA and HFIP. The 100% A solvent system shows an order of magnitude less metal ion leaching than the UV solvent system. It is unclear as to which component in this mixture is responsible for the decrease or if they act in coordination with one another. This will be investigated in future experiments. Once again, the coated components show far less metal introduction across all solvent concentrations.

The amount of metal ion leaching seems to have an impact on the recovery of oligonucleotides as seen in a brochure from YMC for their Dursan-coated line of HPLC columns. The experiment is performed on 50 x 2.1 mm ID HPLC columns without any silica packing material in the column hardware. While the conditions are not identical to this study, they are very close. When the 20mer is in both the Mass Spec (TEA+HFIP) and UV (TEAA) solutions, the recovery is below 100%. The UV solution, which showed more metal ion leaching in this study, shows poorer recovery. It only reaches 34% recovery even after 20 injections. The mass spec solution is significantly better reaching 86% recovery after 20 injections. The coated hardware shows 100% recovery of the oligonucleotide from the very first injection. This indicates that good recovery of oligonucleotides is possible once metal is removed from the flow path.

Conclusion

Nucleotide-based therapeutics have garnered a great deal of interest over the past two decades. Along with that interest comes the need for the accurate analysis of these compounds. Ion-pairing reverse phase is a popular mode for analysis via HPLC, but little work has been done to investigate the impact of these solvent systems on stainless steel surfaces. While it may seem they do not visually corrode steel like other solvents might, they can leach trace amounts of metals into the flow path that can be deleterious to separations. Here we show that a UV compatible solvent additive of TEAA increases the metal ion leaching effect while a mass spec compatible solvent system of TEA and HFIP decreases the amount of metal ions in solution. This has a direct impact on the recovery of oligonucleotides. Dursan coated metal can not only stop metal ions from entering the solvent, but also eliminate chelation sites on the surfaces of frits and column tubes allowing for 100% the recovery of the oligonucleotide of interest.

References:

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