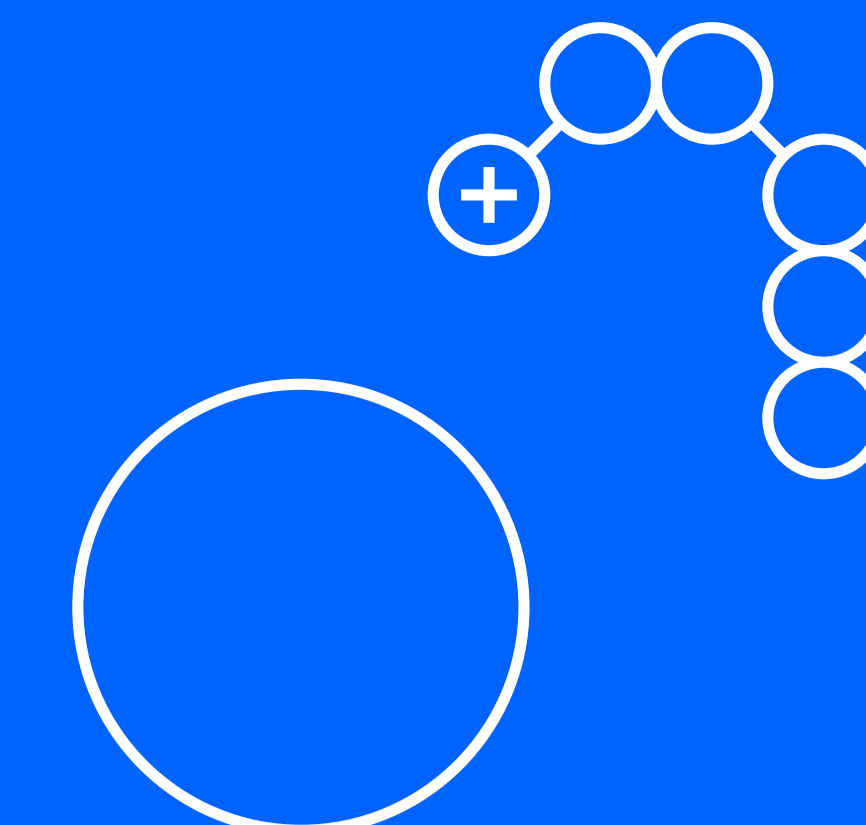


Conversion of ion-pairing agents/counter ions for oligonucleotides using AIEX

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Introduction

Efficient purification of therapeutic molecules is of the utmost importance. Equally important, is the removal of residuals, such as ion-pairing agents (IP agents), used in the purification processes. IP agents are used to improve the separation of hydrophilic and polar molecules in reversed phase chromatography (RPC), also known as ion pair chromatography. Such separation techniques are primarily being used to purify peptides and oligonucleotides. IP agents being amines and acidic counterions interact very tightly with the target molecules and are thus difficult to remove. Many different removal strategies have been employed for different combinations of targets and IP agents. Ion exchange chromatography (IEX) is one such method. Here the principal, advantages and disadvantages, and operational protocols when applying IEX for IP removal from oligonucleotides are described.

Purpose of IP agents

- IP agents associate with the oligonucleotides via electrostatic interactions → rendering them inert → increasing their affinity to the hydrophobic RPC ligands
- Needed for optimal separation of polar species
- Cationic IP agents are used in oligonucleotide purifications
- Often needs to be converted to another more biocompatible ion
- IP agents are difficult to remove since they tightly interact with the target

Commonly used IP agents in oligonucleotide and peptide purifications

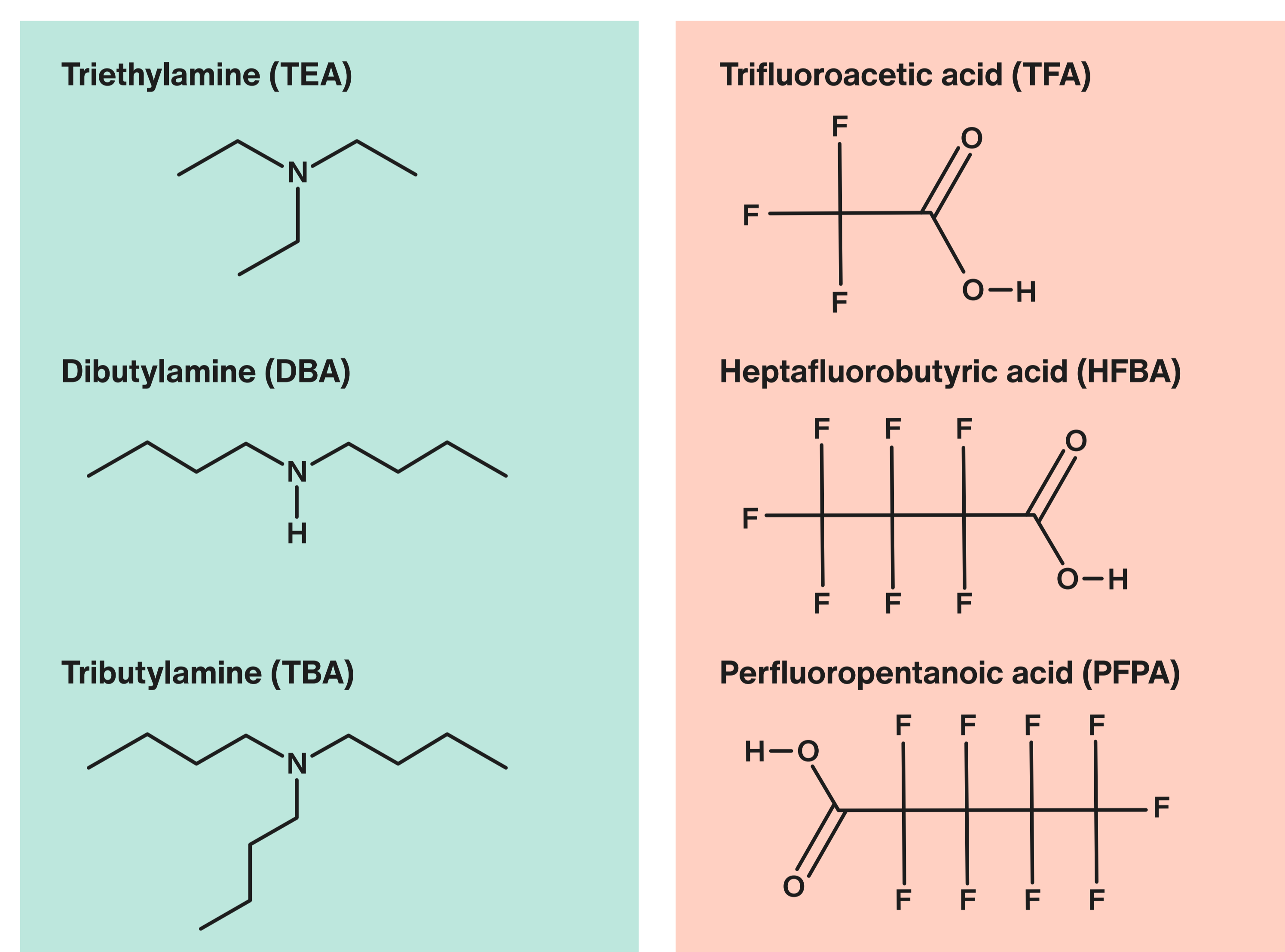


Figure 1. Commonly used IP agents for oligonucleotide (green) and peptide (pink) RPC/IPC purifications.

Removal of IP agents

There are different approaches that can be used to remove IP agents, including chromatographic strategies using IEX and RPC, where either the target molecules or the IP agent can be bound to the resins for exchange of counter ion. See Table 1.

Table 1. Examples of different chromatographic IP agents exchange strategies that can be considered.

Strategy	In practice
IEX – binding of target	Works well at reduced flow rates, see a more detailed description to the right.
IEX – binding of IP agent	Difficult to reach equilibrium/binding of IP agents unless done in batch mode.
Desalting	Not possible due to too slow conversion rate.
Replace IP agent with a stronger acid	Works, but a stronger acid than IP agent is required which can chemically affect the target molecule and its activity. Limitations of available counter ions for the conversion.

Capturing IP agents with anion exchange chromatography (AIEX)

AIEX can be used to capture the target molecules on the stationary phase while exchanging the counter ions/IP agents. It is important to remember that the interactions between the anionic oligonucleotide target and the positively charged ligands will require some time since the IP agents are blocking many of the interaction sites on the oligonucleotide. Therefore, a slow flow rate must be applied during the loading phase of the target molecule. Oligonucleotides have a low isoelectric point (pI) and for that reason the IP agents can be converted using neutral conditions. Moreover, it is important that the ionic strength must not be too high to facilitate ionic interactions and also remember that a final desalting step may be needed after the target molecule elution (Figure 2). Below follows a protocol for DBA conversion to sodium ions using AIEX.

Protocol for DBA-Sodium conversion for oligonucleotides using AIEX

1. Pack a column with WorkBeads™ 40Q (AIEX)
2. Perform a counter ion loading step (3–5 column volumes (CV)) of the resin by running a high concentration of chloride, e.g. 2–3 M NaCl
3. Equilibrate the column with your binding buffer including sodium using e.g. 10 mM NaCl
4. Apply the oligonucleotide to the column at a low flow rate (residence time of e.g. 5–10 minutes). Load of ~40–50 mg oligo/mL resin is recommended
5. Wash the oligo ~10 CV or longer with binding buffer to ensure conversion of DBA to sodium (can be done at a higher flow rate)

Note that if thiophosphorylated (PS)-oligonucleotides are applied, ~10–30% acetonitrile might need to be added to the mobile phases to increase solubility and to prevent unspecific hydrophobic interactions with the resin.

In order to decrease the elution volumes of PS-oligonucleotides, use NaCl molarities in the higher range. Best option is to use a chaotropic elution salt, such as NaBr or NaClO₄ to reduce hydrophobic interactions for improved elution efficiency.

IP conversion DBA⁺ → Na⁺

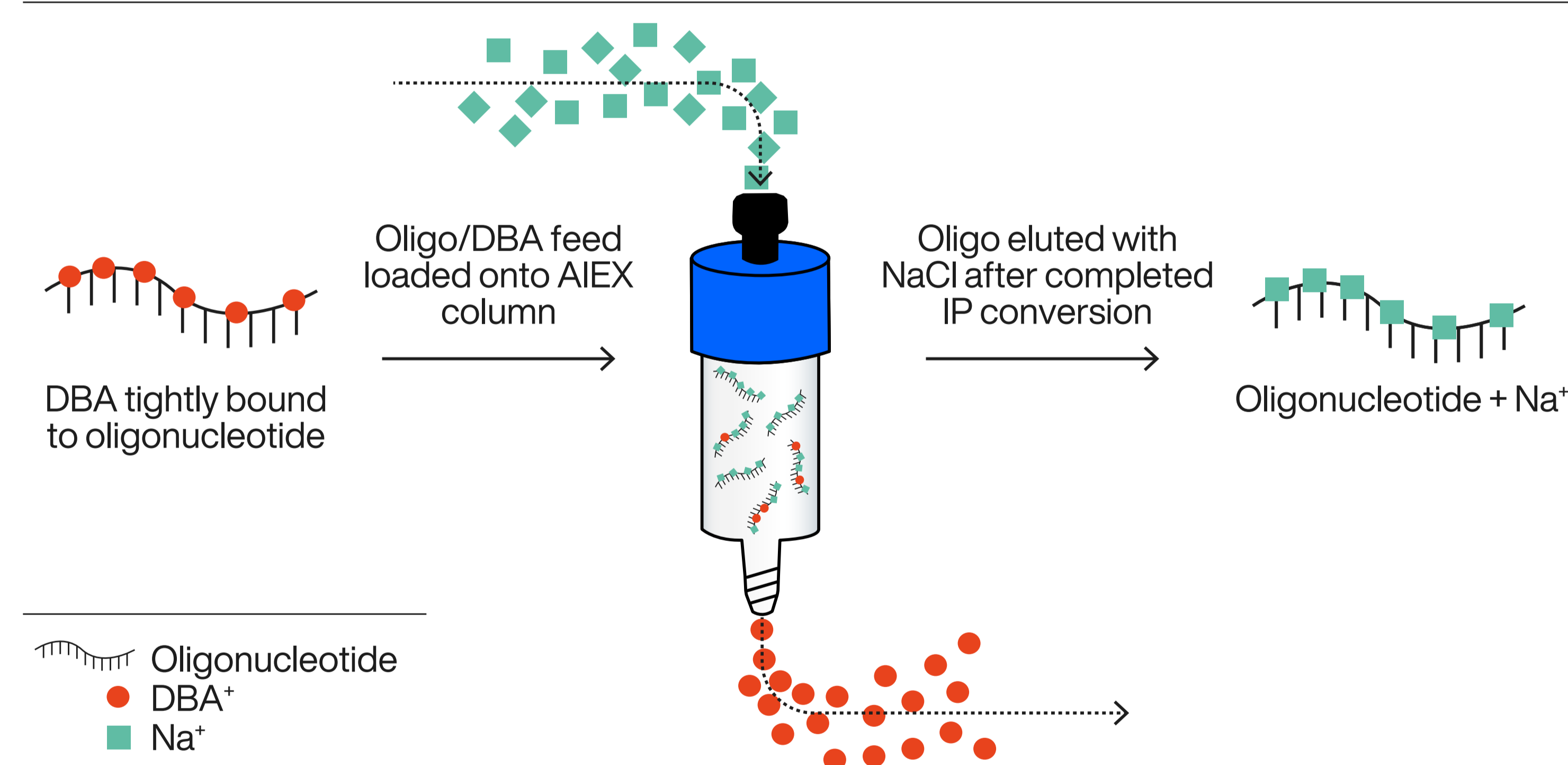


Figure 2. Illustration of conversion of IP agents/counter ions using AIEX in oligonucleotide purification.

Conclusions

Toxic or interfering IP agents originating from oligonucleotide processes can be converted to more biocompatible counter ions using AIEX.

It is important to remember to

- Bind the target molecule to the IEX resin ligand and not the IP agent.
- Use a reduced flow rate during the binding phase for optimal conversion.
- Work with optimal pH and ionic strengths in the mobile phases.
- Increased ionic strength in the elution buffer leads to smaller elution volumes of the target.
- An additional desalting step may be required after the IP agent/counter ion conversion.