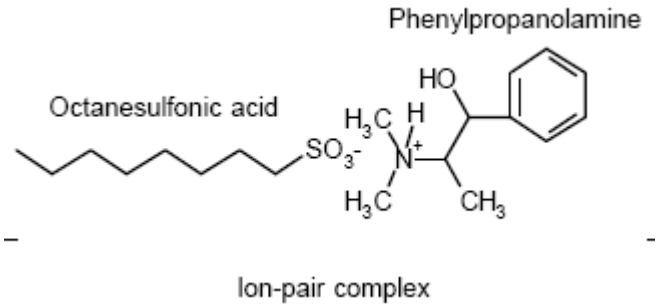
The use of ion-pair reagents can be a handy tool in improving retention and peak shape of ionizable compounds in HPLC (typically reversed phase). Here's how:

Overview

Ion-pairing agents are compounds that contain both an ionic functional group and a hydrophobic portion, such as a hydrocarbon chain. The most common ion-pairing agents are sulfonic acid derivatives such as hexane-, heptane-, octane-sulfonic acids, quaternary ammonium salts such as tetramethyl- or tetrabutylammonium hydroxide, and volatile agents such as trifluoroacetic acid and triethylamine.

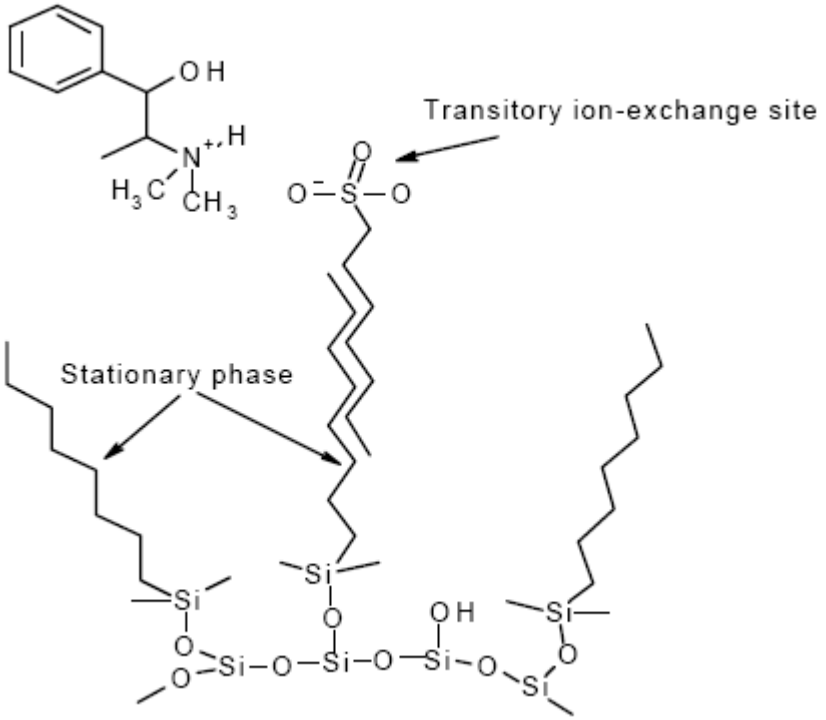
Interaction Mechanisms

As the name implies, ion-pairing agents may exert their principle effect by interacting with any counter-ions in solution, thus forming pseudo-neutral complexes:



Ion-pair complex

Another possible type of interaction can relate to the hydrophobic portions of the ion-pairing agents partitioning into the alkyl bonded phase of the column, forming transitory ion-exchange sites:



Common Drawbacks

It is sometimes unclear which mechanism is the true or primary occurring, and in most cases it is likely a combination of both in various degrees. This could partly relate to the notorious reputation of some ion-pairing methods yielding inconsistent and irreproducible results. Lengthy equilibration and general mass spec incompatibility are among some of the other commonly noted drawbacks. The additional cost of the ion-pair reagent itself may also be a subtle disadvantage that is frequently overlooked.

New Technologies

Recent developments in column technology can in some cases overcome the common drawbacks of ion-pair reagents while meeting the chromatographic challenges:

1. pH stable stationary phase technology can allow for improved retention and

peak shape of basic analytes by running at basic mobile phase pHs controlled with buffers instead of utilizing ion-pair reagents.

2. Hydrophilic Interaction Liquid Chromatography (HILIC) stationary phases are improved.
3. HILIC method development techniques can allow for improved retention of polar analytes under mass spectrometry compatible conditions.

Ideal Ion-Pairing Scenarios

While some of the utility of ion-pairing solutions may be usurped by newer technologies and techniques, there still remain cases where ion-pairing remains the most useful:

1. Samples with a wide range of polar, non-polar, and ionizable compounds that would not otherwise be characterized under the same conditions
2. Ionized compounds lacking a chromophore for UV detection (some ion-pair conjugates absorb UV where the analyte itself would not)
3. Method development familiarity and abundance of literature to reference and extrapolate.

Related resources:

- Ion-Pairing pH 8.3 LC/MS Oligonucleotides
- Trityl-on RNA and DNA Purification
- Basic Compounds by LC/MS Using a High pH Mobile Phase
- Low Molecular Weight Separation by GFC

- Synthetic Oligonucleotides by LC/MS
- pH LC Poster
- HPLC Column Care Guide

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