

Ion pair chromatography: a critical prespective

Abstract

Reversed phase chromatography is one of most popular modes of chromatography in use. Ion pair chromatography a subset of reverse phase chromatography even though happens due to ionic interactions, needs a reverse phase stationary phase as support. Retention of basic analytes on reverse phase columns is the most commonly encountered situation where ion pair chromatography is used. Reproducibility problems in gradient elution, slow equilibration times are some of the disadvantages of ion pair chromatography for which mixed mode columns can provide the best possible solution. In this concise review, an attempt has been made to give an overview of all aspects of ion pair chromatography.

Keywords: ion pair chromatography, ion-pair agents, octane sulfonic acid, trifluoroacetic acid, mixed mode columns

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Introduction

There is no exaggeration in saying that High Performance Liquid Chromatography has helped the pharmaceutical industry to grow by leaps and bounds.¹ Most workers using reverse phase chromatography prefer silica based columns in the low pH range. Silica based columns available today are very robust in the pH range 2 to 8. Since most of the reversed phase chromatography is run in the pH between 2 to 3, these conditions are best suitable for the retention of acidic molecules. Neutral molecules would show significant retention irrespective of pH. But the issue is with the basic compounds which cannot be retained on the column successfully because they remain ionized at acidic pH. In cases where samples have both acids and bases ion pairing might be a best option.

What is ion pairing?

Reverse phase separation is a result of interaction between non polar molecules and non-polar stationary phase. Retention can be adjusted by varying the organic percentage of the mobile phase. Most non polar molecules and acidic molecules due to low pH of the mobile phase can be made to retain well on the column for a good quality separation. However basic molecules which remain ionized at low pH are the ones that are hard to retain. This is where the ion-pair agents² come in handy. Basically ion pair reagents have a polar head and a non-polar tail made of long carbon chains. One example is octane sulfonic acid. The non-polar end gets attracted to non-polar stationary phase leaving its charged end hanging in the mobile phase. The basic species can now get attracted to the oppositely charged immobilized ion pair reagents thus causing chromatographic retention³ (Figure1). The pairing may also happen in the bulk of the mobile phase which gives rise to a neutral molecule that interacts with the non-polar stationary phase. So a negatively charged ion pair reagent can be used to retain positively charged basic molecules.

Alternative approaches

Some of the alternative approaches to ion pair reagents are using

- Trifluoroacetic acid⁵
- Mixed mode columns.

Trifluoroacetic acid is a commonly used ion pair reagent nowadays for biological samples like proteins and peptides.⁶ Trifluoroacetic acid also equilibrates faster and can be used with gradient elution.

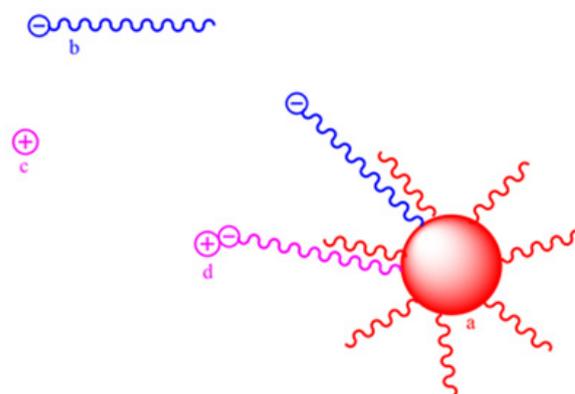


Figure 1 Schematic of ion pair chromatography a) Silica partile with bonded phase. b) Ion pair reagent in free solution. c) Basic ion analyte in free solution. d) Basic ion analyte retained by ion pairing.

Downsides of ion pair chromatography

Gradient elution is a difficult task in ion pair chromatography.⁴ Slow equilibration between mobile phase and column is another issue. For example a 150x4.6mm column might need up to 1mmol of ion pair reagent for complete coverage by an ion pair agent. At a concentration of 5mM, it may take at least 200ml to load up the column. At a flow rate of 1ml/min it may take up to 4 hours for equilibration. Also ion pair reagents due to strong hydrophobic interactions cannot be completely flushed from the column even after extensive washing. This means that dedicating a column to a particular ion pair application is very important. Since ion pair reagents show significant absorbance in the lower UV region, method development in lower UV wavelength region might be a difficult task due to interferences in absorbance from the mobile phase components.

Today's chromatography market is flooded with different kinds of mixed mode columns⁷ which are a convenient alternative to ion pair chromatography. For example polar embedded groups⁸ have a positive or negative charge at the base of carbon chain which provide both ionic and reverse phase character and can possibly eliminate the need of an ion pair agent.

Conclusion

Even though ion pair chromatography has its shortcomings in some cases it might give you the best possible solution. Make sure to

give enough time for equilibration when using ion pair reagent based mobile phases. Try to explore other options like using trifluoroacetic acid, mixed mode columns etc.

Acknowledgments

None.

Conflicts of Interest

None.

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