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Review

Hydrophilic interaction chromatography

Separation of polar compounds on polar stationary phases with partly aqueous eluents is by no means a new separation mode in LC. The first HPLC applications were published more than 30 years ago, and were for a long time mostly confined to carbohydrate analysis. In the early 1990s new phases started to emerge, and the practice was given a name, hydrophilic interaction chromatography (HILIC). Although the use of this separation mode has been relatively limited, we have seen a sudden increase in popularity over the last few years, promoted by the need to analyze polar compounds in increasingly complex mixtures. Another reason for the increase in popularity is the widespread use of MS coupled to LC. The partly aqueous eluents high in ACN with a limited need of adding salt is almost ideal for ESI. The applications now encompass most categories of polar compounds, charged as well as uncharged, although HILIC is particularly well suited for solutes lacking charge where coulombic interactions cannot be used to mediate retention. The review attempts to summarize the ongoing discussion on the separation mechanism and gives an overview of the stationary phases used and the applications addressed with this separation mode in LC.

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1 Introduction

LC was discovered [1 – 3] by observation of colored compounds being separated on polar sorbents such as cellulose fibers or inorganic oxides, and eluted with nonpolar organic solvents. During the first century or so of its existence, this was the "normal" mode of operating a liquid chromatographic setup. When bonded phase columns started to appear in the 1970s, it was soon realized that a system of opposite polarity, *i.e.*, with a hydrophobic stationary phase and aqueous solutions of water-miscible organic solvents offered substantial advantages. The main drawbacks of normal phase HPLC on naked inorganic oxides were slow equilibration and a site heterogeneity that resulted in nonlinear isotherms, which in turn means peaks that are tailing/fronting and shift their retention times with concentration of the injected substance. Another factor that promoted the popularity of "reversed" phase (RP) HPLC was its suitability for a large fraction of the bioanalytical solutes that were of interest. Pharmaceutical development has since long involved a screening of the water/octanol partitioning [4], which is related to the transport, absorption, and distribution of chemicals in biological systems, and hence the pharmacodynamic properties [5]. It turns out that most useful drugs have traditionally been found in a polarity interval that allows them to be well separated from most naturally occurring substances in blood plasma on an octadecyl-bonded phase, using as eluents aqueous buffers with a water-miscible solvent. The tremendous popularity of RP-HPLC left naked inorganic oxides behind, and solution phase chemistries such as micellar chromatography [6] were developed to allow separation of excessively retained compounds on regular RPs with eluents of suitable composition.

A problem that has been the focus of much attention is how to create retention in RP-HPLC for compounds with no or very low inherent distribution toward packings with conventional RP functionality. In those cases where some retention is seen, it usually calls for eluents with very low admixtures of organic solvents. Inadequate phase wetting and expulsion of eluent from the pore space often accompanies such efforts [7] and highly aqueous eluents are therefore often connected with nonrepro-

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Abbreviations: **AEPD,** 2-amino-2-ethyl-1,3-propanediol; **HILIC,** hydrophilic interaction chromatography; **NP,** normal phase; **NPC,** normal phase chromatography; **PSP,** paralytic shellfish poisoning; **S-DVB,** styrene-*copoly*-divinylbenzene; **TEAP,** triethylammonium phosphate