



Ion-exchange and hydrophobic interactions affecting selectivity for neutral and charged solutes on three structurally similar agglomerated ion-exchange and mixed-mode stationary phases



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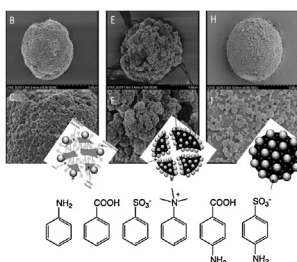
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HIGHLIGHTS

- Explores mixed-mode retention mechanism on agglomerated particles.
- Demonstrates mixed-mode capabilities of related agglomerated phases.
- Provides better understanding of retention mechanisms in play.
- Demonstrates significant selectivity changes on mixed mode phases.
- Determines hydrophobicity of related agglomerated phase materials.

GRAPHICAL ABSTRACT



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ABSTRACT

The nature and extent of mixed-mode retention mechanisms evident for three structurally related, agglomerated, particle-based stationary phases were evaluated. These three agglomerated phases were Thermo Fisher Scientific IonPac AS11-HC – strong anion exchange, Thermo Fisher Scientific IonPac CS10 – strong cation-exchange PS-DVB, and the Thermo Fisher Scientific Acclaim Trinity P1 silica-based substrate, which is commercially marketed as a mixed-mode stationary phase. All studied phases can exhibit zwitterionic and hydrophobic properties, which contribute to the retention of charged organic analytes. A systematic approach was devised to investigate the relative ion-exchange capacities and hydrophobicities for each of the three phases, together with the effect of eluent pH upon selectivity, using a specifically selected range of anionic, cationic and neutral aromatic compounds. Investigation of the strong anion-exchange column and the Trinity P1 mixed-mode substrate, in relation to ion-exchange capacity and pH effects, demonstrated similar retention behaviour for both the anionic and ampholytic solutes, as expected from the structurally related phases. Further evaluation revealed that the ion-exchange selectivity of the mixed-mode phase exhibited properties similar to that of the strong anion-exchange column, with secondary cation-exchange selectivity, albeit with medium to high anion-exchange and cation-exchange capacities, allowing selective retention for each of the anionic, cationic and ampholytic solutes. Observed mixed-mode retention upon the examined phases was found to be a sum of anion- and cation-exchange interactions, secondary ion-exchange and hydrophobic interactions, with possible additional hydrogen bonding. Hydrophobic evaluation of the three phases revealed log *P* values of 0.38–0.48,

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suggesting low to medium hydrophobicity. These stationary phases were also benchmarked against traditional reversed-phase substrates namely, octadecylsilica YMC-Pac Pro C18 and neutral μ PS-DVB resin IonPac NS1-5u, yielding $\log P$ values of 0.57 and 0.52, respectively.

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1. Introduction

Over recent years so-called mixed-mode high-performance liquid chromatography (HPLC) has emerged as a possible alternative approach to the use of single-mode chromatographic columns, for the separation of diverse mixtures of charged, polar and non-polar solutes. Several column manufacturers now produce novel chromatographic supports, displaying bi- and tri-modal functionality, primarily targeting the small molecule pharmaceutical analysis sector. To identify the need (and potential significance) for these new mixed-mode phases, it is first important to consider some of the well-documented limitations of the established single-mode HPLC approaches.

Reversed-phase high-performance liquid chromatography (RP-HPLC) is of course the most widely used and most versatile mode of HPLC, which has been developed over the years into the method of choice for the separation of low polarity and hydrophobic molecules. However, RP-HPLC provides limited possibilities for the variation of retention and separation selectivity of highly polar and charged solutes, without recourse to mobile phase additives [1,2]. On the other hand, traditional normal-phase HPLC (NP-HPLC) can be applied to the separation of polar solutes. However, this approach also has significant limitations, such as low solubility of polar solutes and buffers in organic solvents, issues with sample matrix compatibility and irreversible adsorption of some solutes, extended column equilibration times, and perhaps most significant for the pharmaceutical laboratories, an incompatibility with mass spectrometric (MS) detection.

In the early 1990s, hydrophilic interaction liquid chromatography (HILIC) emerged as a potential solution to the longstanding issue of the separation of polar solutes using HPLC. This technique, which utilises mobile phases compatible with aqueous samples, experiences less solubility issues with polar solutes, and exhibits better compatibility when combined with MS, has understandably since seen enormous growth in popularity [1–6]. However, despite growing utility, it is fair to say some uncertainty remains as to exact retention mechanisms at play with HILIC phases, with the possibility, and perhaps probability, of mixed-mode retention actually being responsible for most so-called HILIC separations. In addition, it is clear that HILIC is not appropriate for the separation of hydrophobic solutes.

For fully charged solutes, ion-exchange chromatography (IEC) is the common approach used [7,8]. The large variety of currently available strong and weak anion- and cation-exchangers has seen the application of IEC grow rapidly. Applications now include not only small inorganic and organic ions, but also charged complexes, charged biomolecules (large and small), ionic polymers and surfactants, etc. However, IEC is unsuitable for neutral species, and therefore as with each of the other singular modes of LC mentioned above, this excludes the possibility to simultaneously separate charged and neutral solutes within a single run.

To address the selectivity limitations (or boundaries) of the above techniques, a number of strategies have been developed based on multi-modal or mixed interactions. Connecting columns in series, or within multi-dimensional HPLC systems is a popular approach, although the latter instance requires rather complex instrumental arrangements and can suffer from both mobile phase and column size incompatibility issues [9]. The packing of mixed-bed phases has also been reported, particularly the combination of IEX and reversed-phase substrates. Indeed, Imtakt Corporation

produce a mixed-bed column, consisting of a mixture of two independently modified silica particle phases, marketed under the generic name, Scherzo. The combination of ion-exchange and reversed-phase substrates has seen particular application within the proteomics area [10,11], although this approach has also been successful in regard to the separation of small neutral and charged solutes [12–14].

An alternative approach to mixed particle systems is the use of multi-functional particles or particles modified with multi-functional groups/ligands. For example, in the case of charged solutes, additional variation of separation selectivity, especially for oppositely charged solutes, can be achieved with zwitterionic stationary phases [15].

Agglomerated ion-exchangers can be considered to provide similar possibilities, as each particle possesses within its structure, areas or layers of both positive and negatively charged functional groups. The classical design of such phases encompasses a charged, surface-functionalised core particle (e.g. ethylvinylbenzene-divinylbenzene, aminated or sulfonated), electrostatically holding polymeric nano-particles (latexes) of the opposite charge, which define the properties of the phase as either a predominantly cation- or anion-exchanger. However, as the inner core of the agglomerated particle is also charged, potential for additional ion-exchange interactions, opposite to those expressed by the immobilised latexes, also exists. Indeed, observations on zwitterionic properties of various agglomerated cation- and anion-exchangers were reported by Bruzzoniti and Pietrzyk some time ago [16,17]. More recently, a commercial agglomerated (so-called ‘raspberry like’) mixed-mode phase marketed under the name of Acclaim Trinity P1 has been released by Thermo Fisher Scientific (formerly Dionex). This phase has a core of porous silica containing internally anchored anion-exchange groups, terminal to hydrophobic C₁₀ chains, whilst the outer surface of the silica particle is agglomerated with nano-polymer beads, themselves functionalised with cation-exchange groups [18].

Other new column selectivity resulting from the co-arrangement of various combinations of ion-exchange, RP, HILIC and size exclusion (SEC) sites/functionalities upon or within a single stationary phase substrate has also been reported recently, although primarily for separations in the biopolymer and proteomics areas [3–6,19–24]. In the area of small molecule separations, mixed-mode stationary phases have perhaps seen less utility [25–27]. Along with the above mentioned release of the Trinity P1 column, companies such as SIELC Technologies have produced a new bonded phase based upon a hydrophobic ligand with embedded anion-exchange functionality, and a surface cation-exchange functionality, marketed as Obelisc R [28–30]. An alternative configuration, which includes a hydrophilic spacer functionality is also available under the trade name of Obelisc N [20,31]. In addition, SIELC also market a range of bi-modal phases, under a generic name of Primesep [32–36]. However, considerable uncertainty exists as to the true nature of such phases, their physical and chemical stability, and exact retention mechanisms. The Acclaim Trinity P1 was reported to circumvent some of these issues through its distinctive structural arrangement, whereby ion-exchange groups and reversed-phase sites can operate simultaneously and to some extent in spatial isolation from each other [18], although this inherently unique phase architecture makes characterisation of the exact retention mechanisms occurring for anionic, cationic and neutral solutes rather complex.

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