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Mixed mode HILIC/anion exchange separations on latex coated silica monoliths

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ABSTRACT

Bare silica monoliths do not possess anion exchange sites hence they show low retention for anions. Moreover, bare silica monoliths show low retention in hydrophilic interaction liquid chromatography (HILIC). Coating the silica surface with cationic nanoparticles e.g. AS9-SC (latex A), AS12A (latex B) and DNApac (latex C) increases the thickness of the water layer on the Onyx silica monolith 8–10 times enabling HILIC retention when a high % acetonitrile (ACN) mobile phase is used. The formed water layer by itself is not sufficient to perform good separation of the studied anions (acetate, formate, nitrate, bromate, thiocyanate and iodide). On the other hand, the latex nanoparticles introduce positively charged sites, making anion exchange chromatography possible, with the anion exchange capacity varying with the latex adsorbed (44.1 ± 0.2, 4.4 ± 0.1 and 14.0 ± 0.7 μeq/column for latex A, B and C, respectively). Latex A nanoparticles which provided the highest ion exchange capacity separated all tested anions with reasonable resolution. Fast separation (2.5 min) of acetate, formate, nitrate, bromate, thiocyanate and iodide was performed using the latex A coated silica monolith. The obtained efficiencies are 13,000–50,000 plates/m at 3 mL/min with a minimum resolution of 0.85. Retention is mixed mode under HILIC conditions with HILIC dominating for the kosmotropic anions and ion exchange dominating for the chaotropic anions. The two different brands of silica monoliths (Merck Chromolith and Phenomenex Onyx) coated with the same latex A nanoparticles displayed similar water layer volumes, ion exchange capacity and selectivity.

1. Introduction

Monoliths were introduced as HPLC columns in the late 1980s-mid 1990s [1–3]. A monolith is a single large rod of porous substance that fills the whole column volume without any gaps [4]. Monolithic columns can be prepared either through polymerization of organic monomers [5,6] or through sol–gel technology for titania and silica based monoliths [3]. To date most monolithic columns have been prepared for reversed phase and ion exchange chromatography, and only limited research has been performed on monolithic columns for HILIC [7–10]. This paper explores the use of agglomerated silica monolith columns for mixed mode separations based on HILIC and anion exchange.

Silica based monolithic columns are characterized by the presence of relatively small mesopores (10–25 nm) for retention and large macropores (1–3 μm) for through-flow of mobile phase [6,11,12]. Silica based monoliths are usually used for small molecular weight analytes [13,14]. The highly porous structure of the monolith offers high permeability which allows fast separation of analytes at very high flow rates with minimal backpressures [15–20]. For these reasons, silica based monolithic columns are used in this study. However, silica based monoliths have some disadvantages. They dissolve at pH > 8, are available in only limited column dimensions [21], and can exhibit tailing peaks even for neutral analytes [22].

The surface of bare silica possesses deprotonated silanols which act as cation exchange sites. Thus, silica excludes anions, rather than retains them. Several methods have been explored to convert silica columns into anion exchangers. A reversed phase silica particulate column was converted into an anion exchange column by adding a cationic surfactant to the eluent [23,24]. Alternately, semi-permanent anion exchangers were produced by coating a reversed phase particulate column [25] or monolith [26] with a hydrophobic surfactant such as didodecyldimethylammonium bromide (DDAB). Separations of common inorganic anions on a surfactant coated monolithic columns have been performed in just 15–20 s [26]. However, the DDAB coating gradually leaches from the coated column requiring recoating of the column. Precipitation of DDAB in the column sometimes occurs upon recoating, leading to pressure and reproducibility issues [27].

Agglomerated particles are an alternate approach to produce high efficiency ion exchange columns [17]. In an agglomerated

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Table 1
Characteristics of the studied latex nanoparticles.

Latex acronym	Corresponding Dionex column	Latex diameter (nm)	% Cross-linking	Hydrophilicity
Latex A	AS9-SC	105	20	Medium
Latex B	AS12A	140	0.2	Medium
Latex C	DNApac	151	5	-

column for anion exchange, a stationary phase possessing a negative surface charge is coated with positively charged cationic nanometer sized latices. The alkyl quaternary ammonium functionalities on the latex irreversibly bind to the negatively charged surface. For simplification, AS9-SC, AS12A and DNApac PA200 latex nanoparticles are assigned as latex A, B and C, respectively (Table 1). The Dionex IonPac AS9-SC (latex A), AS12A (latex B) and DNApac PA200 (latex C) commercial columns are examples of latex coated particulate columns where 105 nm, 140 nm and 151 nm diameter latices coat cross-linked ethyl vinyl benzene/divinyl benzene (EVB/DVB) microporous particles, respectively [28]. AS9-SC and AS12A Dionex commercial columns can separate common inorganic anions and oxyhalides in 10–12 min [28]. In addition, the IonPac AS9-SC column can separate polarizable anions such as iodide and thiocyanate in 8–15 min [28]. The DNApac PA200 commercial column shows strong anion-exchange properties and was developed for high-resolution analysis and purification of synthetic and modified oligonucleotides [29]. Recently, cationic latex A nanoparticles were used to convert a bare silica monolith into a stable anion exchange phase [17]. The resultant column was more stable and more efficient than a comparable surfactant coated monolith [17].

Such agglomerated phases can also be used for HILIC separations [20]. In HILIC, a mobile phase containing a large percentage of an organic modifier (usually ACN) equilibrates with a hydrophilic stationary phase. A water layer is formed on the surface of the packing. Analytes partition between this water layer and the mobile phase. In our previous work, the adsorbed hydrophilic latex facilitated the formation of a water layer enabling HILIC retention. A latex A coated silica monolith provided efficient (H=25–110 μm) separation of polar analytes (e.g. benzoates, nucleotides and amino acids) under HILIC conditions [20]. The high permeability of the monolith enabled separation of naphthalene, uracil and cytosine in less than 15 s. Selectivity of cationic analytes (e.g. amino acids) was governed by both hydrophilic partitioning and electrostatic repulsion.

Recently it has been recognized that many HILIC separations are in fact mixed mode in nature [30]. Lindner, Bicker and Lämmerhofer studied retention patterns on different stationary phases and concluded that partitioning alone cannot satisfactorily explain the chromatographic behavior of analytes under HILIC conditions [31–33]. Additionally, Dinh et al. studied the selectivity of different HILIC columns to widen the understanding of interactions taking place in HILIC [34]. Dinh et al. used principal component analysis to group different HILIC columns according to the predominant mechanism of retention involved using carefully selected test probes. For instance, Dinh et al. found that bare silica columns follow adsorption, in contrast to zwitterionic phases where partitioning is the main mechanism of retention [34]. Recently, Lucy et al. re-casted the retention data of Dinh et al. [34] mentioned above and additional 12 columns into simple and easy to understand selectivity plots [35].

The current study builds on our past work to characterize the factors governing HILIC separations on latex coated monoliths. The ion exchange capacity and the amount of the water layer associated with these coated monoliths were measured and compared. Further, the recently reported selectivity plots [35] are used to demonstrate the contribution of partitioning and/or ion exchange to the retention of model anionic analytes on latex coated silica monoliths. Finally, the mixed mode retention is utilized to perform fast simultaneous separation of anions that are both weakly and strongly retained on traditional ion exchange columns.

2. Experimental

2.1. Apparatus

The HPLC system was described previously [20]. Briefly, it consists of: a model 625 LC Waters pump (Waters, Milford, MA, USA); a 6-port Rheodyne model 7125 (Rheodyne, Berkeley, CA, USA) injection valve with a 10 μL loop; and a Lambda-Max Model 481 UV detector (Waters). A model LC-600 Shimatzu LC pump (Shimatzu, Japan) was used for coating each silica monolith with the corresponding latex suspension.

2.2. Reagents and materials

All solutions were prepared in nanopure water (Barnstead, Dubuque, IA, USA). HPLC-grade ACN, naphthalene, $NaBrO_3$ and KSCN were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Methylphosphonic acid, adenine, cytosine and uracil were purchased from Sigma-Aldrich (St. Louis, MO, USA). $NaNO_3$ was from EMD Chemical Inc. (Darmstadt, Germany), NaI from BDH Inc. (Toronto, ON, Canada), sodium acetate from Caledon (Georgetown, ON, Canada) and sodium formate from BDH Inc. (Poole, England). Ammonium acetate was from Anachemia (Montreal, QC, Canada) and benzyltrimethylammonium chloride (BTMA) from Acros Organics (Morris Plains, NJ, USA). Three different latex nanoparticles (latices A–C) were provided by Dionex Corp. (Sunnyvale, CA, USA). Table 1 displays the characteristics of these latices A–C in terms of their particle size, % cross-linking and hydrophilicity. All latex nanoparticles are functionalized with alkyl quaternary ammonium groups. The %ACN quoted in this work represent the volume of the ACN relative to the total volume of the solvents including water and ACN. Methylphosphonic acid and ammonium acetate concentrations are present after addition of ACN. The aqueous phase pH was measured by a Corning combination 3 in 1 electrode (Corning, Big Flats, NY, USA). The pH was adjusted by addition of NaOH for all experiments except the ACN study where triethylamine (TEA) (Fair Lawn, NJ, USA) was used.

2.3. Tested columns and latex coating

The latex coating procedure was as reported previously [17,20]. Briefly, three Onyx (Phenomenex, Torrance, CA, USA) bare silica monoliths (100 mm × 4.6 mm ID) were pre-rinsed with 0.01 M HCl. A 1:10 suspension of the latex particles (Dionex) in 0.01 M HCl was prepared for each latex type (latices A–C) and was rinsed through the column until breakthrough (approx. 5 mL). The column was then rinsed with nanopure water to remove any unretained latex. For comparison, the same procedure was applied to a Chromolith Performance (Merck, Whitehouse Station, NJ, USA) bare silica monolith (100 mm × 4.6 mm ID) using latex A nanoparticles. It is worth mentioning that Merck was the first company to commercialize silica monolithic rods under the brand name, Chromolith. They subsequently licensed Phenomenex to make and sell the same product under the brand name, Onyx [36,37]. Both Onyx [38] and Chromolith [39] share the same characteristics of macropore size (2 μm), mesopore size (13 nm) and surface area (300 m^2/g).

2.4. *Measurement of the ion exchange capacity*

The ion exchange capacity of the coated silica monoliths was determined using the breakthrough method [40]. Briefly, the monoliths were flushed continuously with 20 mM NaCl for 30 min at 1 mL/min to saturate all the anion exchange sites with Cl^-. The excess non-adsorbed Cl^- was removed by flushing with nanopure water. Thereafter, 1 mM $NaNO_3$ solution was pumped into the columns at 1 mL/min until breakthrough was observed at 210 nm. Subtraction of the dead time from the breakthrough time yielded the ion exchange capacity in μeq/column.

2.5. *Measurement of the water layer volume*

Using the Bicker et al. method [33,41], the volume of the water layer under HILIC conditions was determined by measuring the difference in the retention time of toluene (unretained) when the protic modifier was changed from water to methanol [31]. Other details of the method are given in Section 3.1.

3. Results and discussion

As previously mentioned, a latex A coated silica monolith was used for separation of chloride, nitrite, bromide, nitrate, phosphate, iodide and sulfate ions under pure aqueous ion exchange conditions [17]. The latex A coated silica monolith was also utilized for separation of hydrophilic analytes under HILIC conditions [20]. In this study, latices A–C nanoparticles were used to coat three Onyx bare silica monoliths. The latex irreversibly binds to the silica surface converting the bare silica monolith into an anion exchange phase as illustrated in Fig. 1. The electrostatic binding of the polycationic latices to the surface is stable [20].

The mixed mode (HILIC vs. ion exchange) retention characteristics of these different latex coated silica monoliths were assessed with regards to the associated water layer volume, ion exchange capacity and selectivity. This study provided an overall comparison between the two different brands (Chromolith vs. Onyx) when coated with the same latex type in terms of selectivity, water layer thickness and ion exchange capacity. Finally the contribution of partitioning and/or ion exchange to retention of kosmotropic and chaotropic anions was studied.

3.1. *Characterization of latex coated monoliths*

The formation of a water layer on the surface of HILIC stationary phases forms the basis of retention in HILIC [42]. Herein, the Bicker et al. method [33,41] was used to determine the volume of the water layer associated with the surface of a three different latex (latices A–C) coated silica monoliths. The volume of the water layer was determined indirectly by measuring the difference in the retention time of an unretained compound (toluene) when the protic modifier was changed from water to methanol [31]. The retention time of toluene increases because the water layer is replaced by a methanol layer into which toluene partitions strongly. The difference in retention volume corresponds to the volume of the water layer associated with the packing material. Table 2 summarizes the water layer volumes associated with these latex coated monoliths and a bare silica Onyx column. The observed water layer on the monolithic bare silica (0.01 mL) is small compared to that measured for particulate columns (e.g. 0.19 mL for Atlantis HILIC silica [43]). This is consistent with observations that HILIC retention of polar analytes was much smaller on monolithic silica columns compared to particulate columns [44,45]. Indeed silica monoliths showed the lowest retention of the 22 HILIC columns recently studied [34].

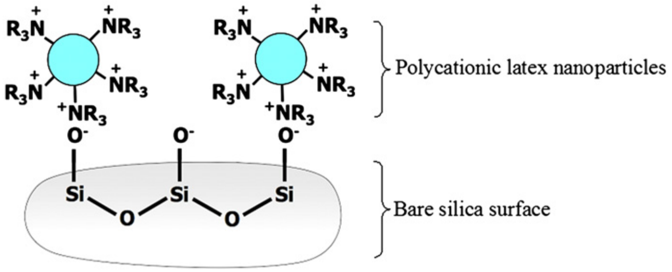

Fig. 1. Schematic of the agglomerated latex coated silica monolith (adapted from Ref [61]).

Some selected anions (acetate, formate, bromate, nitrate, thiocyanate and iodide) were used to test the performance of these latex coated monoliths under HILIC conditions. These anions represent a range of retention characteristics in traditional ion chromatography. In traditional ion chromatography, formate and acetate are weakly retained and thus difficult to discern from the water dip. Conversely, polarizable anions such as iodide, thiocyanate and thiosulfate are strongly retained on ion chromatography columns such as the Dionex IonPac AS9-SC column [28].

As shown in Fig. 2, these anions are not retained on a bare silica monolith under HILIC conditions due to the lack of an adsorbed water layer (Table 2). Anionic analytes would experience further reductions in retention due to the repulsive forces between the negatively charged silanol groups of the bare silica and anion, as per the electrostatic repulsion-hydrophilic liquid interaction chromatography (ERLIC) mode [46]. Hence neither HILIC nor ERLIC separation of these anions can be achieved on a bare silica monolith.

Adsorbing cationic latices onto the silica surface as depicted in Fig. 1 resulted in a substantial increase in the adsorbed water layer (Table 2). The water layer volumes of the latex coated silica monoliths are 8–10 times larger than that of the bare silica monolith before coating. The greater water layer is believed to be due to the additional surface charge and the hydrophilic nature of the cationic sites of the latex. Adsorption of latex A encouraged the formation of a thicker water layer (0.10–0.12 mL) than latex B (0.09 mL) under HILIC conditions, possibly due to the greater hydrophilicity of latex A nanoparticles [47].

Obviously, coating the silica surface with cationic latex nanoparticles also introduces positively charged quaternary ammonium groups (Fig. 1), enabling these coated phases to act as anion exchangers [17]. The ion exchange capacity was measured using the breakthrough method [40] as discussed in Section 2.4. The ion exchange capacity of the latex A coated Onyx silica

Table 2
Water layer volume and ion exchange capacity of latex coated monoliths.

Column	Water layer volume (mL/col ± SD)	Ion exchange capacity (μeq/col ± SD)
Bare silica Onyx	0.01 ± 0.010	
Latex A coated Onyx	0.10 ± 0.006	44.1 ± 0.2
Latex A coated Chromolith	0.12 ± 0.003	43.8 ± 0.8
Latex B coated Onyx	0.09 ± 0.012	4.4 ± 0.1
Latex C coated Onyx	0.08 ± 0.003	14.0 ± 0.7

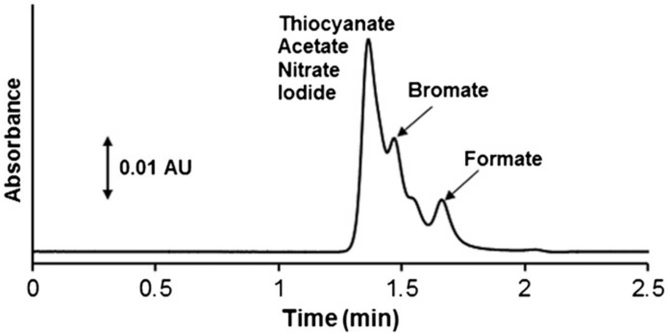

Fig. 2. Separation of acetate, formate, bromate, nitrate, thiocyanate and iodide on Onyx bare silica monolith. Conditions: column, Onyx bare silica monolith (100 × 4.6 mm ID); flow rate, 1.0 mL/min; eluent, 15 mM methylphosphonic acid, pH 4.0, in 65% ACN; analytes, 0.02–14.7 mM in 65% ACN; UV detection at 210 nm with a 10 μL loop injection.

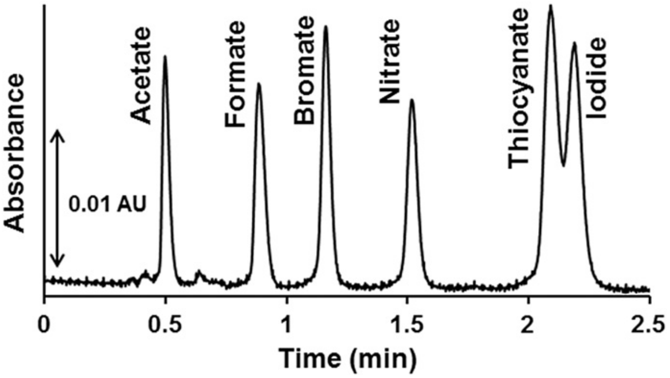

Fig. 3. Fast separation of acetate, formate, bromate, nitrate, thiocyanate and iodide on the latex A coated Chromolith silica monolith. Conditions: column, latex A coated silica monolith (100 × 4.6 mm ID); flow rate, 3.0 mL/min; eluent, 25 mM methylphosphonic acid, pH 4.0, in 65% ACN; analytes, 0.025–7.352 mM in 65% ACN; UV detection at 225 nm with a 10 μL loop injection.

monolithic (44.1 ± 0.2 μeq/column) is comparable to that of a Chromolith silica monolith coated with the same latex A (41 μeq/col.) [17] and is higher than that of the much longer commercial Dionex AS9-SC column (35 μeq/col., 250 mm × 4.0 mm ID) [47]. In contrast, the observed capacities of the latices B and C coated monoliths are substantially smaller than those of the corresponding commercial columns (52 and 40 μeq/col., respectively). The higher capacity of the latex A coated monolith correlates with the higher degree of cross-linking (20%) of latex A compared to the other latices e.g. latex B (0.5% cross-linked) and latex C (5% cross-linked) as shown in Table 1. The high degree of cross-linking of latex A allows these nanoparticles to bind to the silica surface as district ion exchange sites, i.e., the ion exchange sites are completely exposed and available for ion exchange interactions on the silica surface leading to higher ion exchange capacity. In contrast, latex nanoparticles with low degree of cross-linking e.g. latices B and C would spread out the ion-exchange sites leading to reduction of the number of these sites per unit area. This is consistent with what has been reported previously that the use of latex nanoparticles with low degree of cross-linking yielded low ion exchange capacity of the coated resin [48].

3.2. Fast HILIC separations on latex coated monoliths

As noted earlier, in traditional ion chromatography formate and acetate are weakly retained and thus difficult to discern from the water dip. Conversely, polarizable anions such as iodide, thiocyanate and thiosulfate are strongly retained on ion chromatography columns such as the Dionex AS9-SC column [28]. Fig. 3 shows that under HILIC conditions on the latex A coated monolith, both acetate and formate are retained sufficiently to separate them from the dead time marker, while still eluting iodide and thiocyanate in reasonable time.

Although all of the latex coated monoliths in Table 2 have similar water layers, only the latex A coated column was able to separate all fore mentioned anions (resolution between thiocyanate and iodide of 0.85). On the latices B and C coated monoliths (Figs. S1 and S2), iodide and thiocyanate eluted together as a single peak and baseline resolution was not obtained between formate and bromate. This indicated that the retention of anions on the latex coated silica monoliths does not solely follow HILIC mechanism. Rather, the ion exchange nature of the column is also critical. The high ion exchange capacity of the latex A coated monoliths may contribute to the superior separation performance of this column relative to the other latex coated columns (latices B and C).

The monolith silica support provides the latex coated column with high permeability allowing separation to be performed at very high flow rates with minimal backpressure [15–20]. Fig. 3 shows a 2.5 min separation of some common UV absorbing anions on the latex A coated silica monolith under HILIC conditions at 3 mL/min. Higher flow rates resulted in decreased resolution between thiocyanate and iodide, such that by 8 mL/min only a single peak was observed. The separation in Fig. 3 is comparable in speed to that achieved on a 10 cm long zwitterionic lysine bonded silica monolith (~1.8 min) [49] for anions ranging from NO_2^- to SCN^-. Fig. 3 is slower than the 1.5 min ion exchange separation achieved on a 0.5 cm long DDAB coated silica based monolith [18] and the 30 s on a 5 cm DDAB coated RP monolith [26]. However the anions separated in this past work did not include either kosmotropic anions such as acetate or formate nor chaotropic anions such as SCN^- or I^-.

3.3. Selectivity of latex coated silica monoliths

Since HILIC was coined by Alpert in 1990 [42], there has been a dramatic increase of the number of HILIC stationary phases including bare silica, amide, zwitterionic, amine and diol phases [50,51]. Substantial differences in selectivity have been observed between various types of HILIC phases [35,50,51]. These differences in selectivity indicate that interactions including hydrophobic, hydrophilic, dipole–dipole, hydrogen bonding and/or ion exchange are important in HILIC [34,50]. Recently, Dinh et al. [34] characterized these interactions in 22 commercial HILIC columns using principal components analysis (PCA). Dinh et al. were successful in classifying commercial HILIC phases into 4 main classes: zwitterionic, neutral, cationic and anionic phases [34]. More recently, Lucy et al. re-casted the retention results given by Dinh et al. into simpler selectivity plots [35]. Results displayed by Lucy et al. highly agreed with the column classifications of Dinh et al. The selectivity plot given by Lucy et al. [35] graphed the retention ratio of benzyltrimethylammonium (BTMA)/cytosine on the *Y*-axis vs. the retention ratio of cytosine/uracil on the *X*-axis. BTMA/cytosine pair was used to probe ion exchange interactions, i.e., the higher the BTMA/cytosine retention ratio, the stronger the cation exchanger character [34,35]. A BTMA/cytosine retention ratio of ~1 was indicative of a column without ion exchange character (e.g., zwitterionic and diol columns). A low BTMA/cytosine retention ratio (< 1) indicates anion exchange character. The retention ratio of cytosine/uracil was used to probe hydrophilic interactions, i.e., the higher cytosine/uracil ratio, the stronger the hydrophilic interactions [34,35].

Herein the same probes were used to characterize the selectivity differences among the three latices (latices A–C) coated

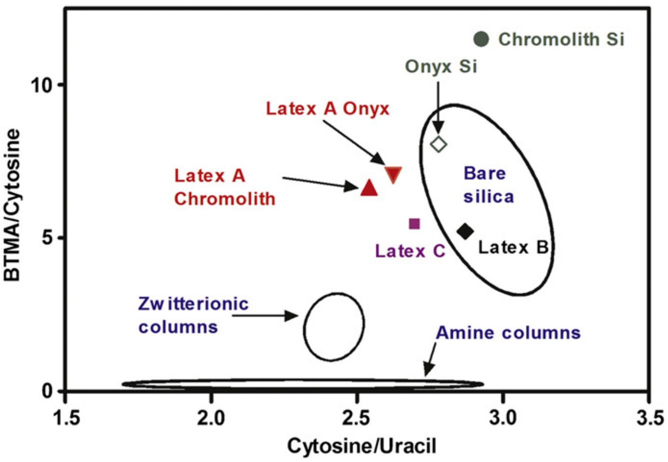

Fig. 4. Selectivity plot of latex coated silica monoliths. Conditions: columns; Onyx bare silica (◇), Chromolith bare silica (●), latex A coated Onyx (▼), latex A coated Chromolith (▲), latex B coated Onyx (◆) and latex C coated Onyx (■); eluent, 5 mM ammonium acetate (pH 6.8) in 80% ACN; test analytes, 0.044–0.44 mM in 80% ACN; UV detection at 254 nm with a 10 μL loop injection. The 75% confidence ellipses around bare silica, amine and zwitterionic columns were adopted from ref [35].

Onyx silica monoliths and a latex A coated Chromolith (Fig. 4). For reference, the 75% confidence ellipses for the various classes of commercial HILIC columns are shown in the figure (e.g. bare silica, amine and zwitterionic phases).

Underivatized silica phases showed strong cation exchange properties as shown in Fig. 4 due to the presence of free silanols. The bare Chromolith monolith displayed unusually strong cation exchange character as it is above the ellipse encompassing typical (75% confidence interval) silica character. This is consistent with the observations of Dinh et al. [34]. The bare Onyx monolith showed ion exchange and hydrophilic character that was typical of bare silica phases.

Coating the monolithic columns results in a decrease in the cation exchange character of the silica monolith (i.e., lower BTMA/cytosine value). However the latex coated columns did not display anion exchange character (BTMA/cytosine < 1) such as observed for commercial amine based HILIC columns. With the exception of the latex B coated column, the latex columns displayed lower hydrophilicity than the bare silica (i.e., are located to the left of the bare silica confidence ellipse in Fig. 4. The result is the latices A and C coated monoliths display unique selectivity amongst HILIC columns.

Chromolith and Onyx bare silica monoliths displayed very similar cation exchange and hydrophilic character when coated with the same latex A (Fig. 4). These columns also displayed similar water layers (Table 2). This is despite the bare Chromolith and Onyx silicas displaying different character. Thus the character of the latex coated monolith is determined by the nature of the latex rather than the underlying silica. Coating an Onyx silica monolith with latex B substantially decreased its cation exchange character. However the resultant retention characteristics were not statistically different from that of other HILIC bare silica columns. This behavior might be attributed to the very low coverage of latex B nanoparticles on the silica surface, consistent with the low ion exchange capacity (4.4 ± 0.1 μeq/col) given in Table 2. The behavior of latex C coated monolith was intermediate between latices A and B columns. The trend in behavior again correlates with the degree of cross-linking of the latex, rather than their size.

3.4. *Influence of the anion nature on the mixed mode retention mode*

Section 3.3 has demonstrated that retention on the latex monolith is mixed mode in nature. In this part, we explore the effect of buffer strength and %ACN of the mobile phase affected the dominant retention mechanism (i.e., partitioning or ion exchange) for anions of widely different character.

3.4.1. *Effect of buffer strength*

A partitioning retention mechanism, as originally postulated by Alpert for HILIC [42] would follow the relationship [51]:

$$\log k = \log k_w - S\varphi_B \tag{1}$$

where φ_B is the volume fraction of the strong solvent, k_w is the retention factor when the mobile phase contains no strong solvent, and S is the slope of the plot. Thus a plot of log k vs. (volume fraction of the solvent) should yield a straight line for a partitioning mechanism. Increased retention with the increasing buffer concentration has also been reported for a variety of HILIC stationary phases [31,41,52–54]. It has been hypothesized that increasing the buffer concentration results in a higher concentration of solvated ions in the water rich layer [31,41,52–54].

On the other hand, ion exchange chromatography is described by [55,56] as follows:

$$\log k_A = C_1 - (x/y)\log[47] \tag{2}$$

where k_A is the retention factor of the analyte anion, C_1 is a constant, x is the charge of the analyte anion, and y is the charge of the eluent anion. Thus a plot of log k_A vs. log (eluent [47]) should yield a straight line for an ion exchange mechanism.

In this study, the retention mechanism was investigated for a number of anions on the latices A–C coated silica monoliths. As shown in Fig. 5, log k for kosmotropic (acetate and formate), intermediate (nitrate) and chaotropic (iodide) anions on the latex A coated monolith were plotted vs. log [buffer strength] resulted in linear relationships with a negative slope ($-x/y$ as described in Eq. (2)). Comparable linearity was observed for thiocyanate and bromate (Supplementary Fig. S3). According to Eq. (2), the slope of log k vs. log (eluent [47]) is equal to the negative ratio of the analyte and eluent charges [56]. Therefore, the slope of this linear relationship of monovalent anions should equal −1.0 under pure ion exchange conditions. Herein the slope of bromate (−0.92), nitrate (−0.86), thiocyanate (−0.87) and iodide (−0.87) are smaller than the theoretical slope (−1.0) expected for ion exchange of monovalent anions on the latex A coated monolith. Thus the linearity and slopes in Fig. 5 indicate that ion exchange is the dominant retention mechanism for many anions.

However, some positive curvature was observed in Fig. 5, suggesting the presence of other competing retention mechanism such as partitioning. This is especially true for acetate and

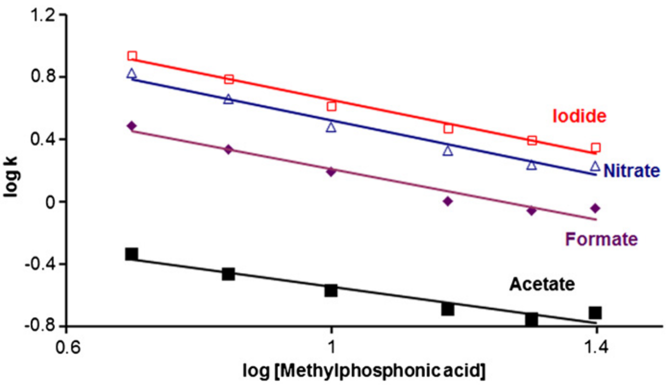

Fig. 5. Effect of buffer strength on the retention of anions on the latex A coated Onyx silica monolith. Conditions: column, latex A coated silica monolith (100 × 4.6 mm ID); flow rate, 1.0 mL/min; eluent, 5–25 mM methylphosphonic acid, pH 4.0, in 65% ACN; analytes, 0.02–14.7 mM acetate, formate, nitrate and iodide in 65% ACN; UV detection at 210 nm with a 10 μL loop injection. Lines are fit to Eq. (2). The size of the data points is equal or bigger than the *Y* error bar.

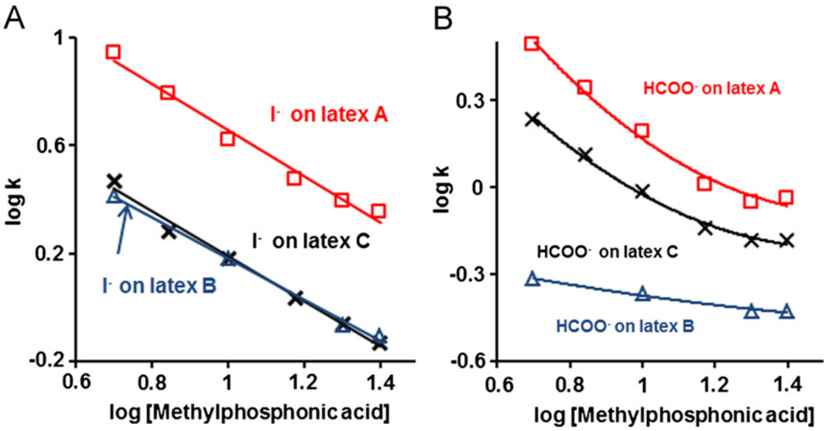

Fig. 6. Effect of buffer strength on the retention of (A) iodide and (B) formate on latex coated Onyx silica monoliths. Conditions: columns, latex A (□), latex B (△) and latex C (×) latex coated Onyx silica monoliths (All, 100 × 4.6 mm ID); flow rate, 1.0 mL/min; eluent, 5–25 mM methylphosphonic acid, pH 4.0, in 65% ACN; analytes, 0.1–14.7 mM formate and iodide in 65% ACN; UV detection at 210 nm with a 10 μL loop injection. Line of Fig. 6(A) is fit to Eq. (2). The size of the data points is bigger than the *Y* error bar.

formate (R^2~0.95) on the latex A coated silica monolith. Moreover, acetate (−0.54) and formate (−0.79) exhibit much smaller slope values, again suggestive that partitioning is involved to a greater extent in the retention of kosmotropic anions. Comparable behavior was observed for other latex coated monoliths including latices B and C. This is not surprising as acetate and formate are weakly retained by typical ion exchangers, while thiocyanate is strongly retained by ion exchange [28,57].

Fig. 6 compares the retention of iodide (A) and formate (B) vs. eluent strength on all three latex coated monoliths. Fig. 6(A) shows a decreasing linear relationship for retention of iodide (R^2~0.99) indicating that ion exchange constitutes a major retention mechanism for all three latex coated silica monoliths. Moreover, the overlap of iodide retention on both latex B (Δ) and latex C (×) coated monoliths (Fig. 6) was highly consistent with their similarity in cation exchange selectivity in Fig. 4. This consistency agrees with that ion exchange is the dominant retention mechanism of chaotropic anions e.g. iodide. Comparable linearity was observed for thiocyanate, nitrate and bromate, as shown in Supplementary Fig. S4. The slope of bromate (−0.66), nitrate (−0.72), thiocyanate (−0.73) and iodide (−0.76) are smaller than the theoretical slope (−1.0) expected for ion exchange of monovalent anions on the latex B coated monolith. On the other hand, Fig. 6(B) displayed positive curvature for formate on all latex coated monoliths indicating the presence of partitioning as a competing retention mechanism for kosmotropic anions. The lower slope for the latex C (−0.62) and latex B (−0.17) columns in Fig. 6(B) are indicative that retention on these columns is dominated by partitioning. Comparable curvature was observed for acetate on the latex B coated monolith as shown in Supplementary Fig. S4.

3.4.2. *Effect of %ACN*

Fig. 7 shows the effect of %ACN on retention of anions on the latex A coated silica monolith. In this study, TEA was used to adjust the pH of methylphosphonic acid due to its high solubility in ACN rich mobile phases [42]. Consistent with the results above, the retention behavior of acetate and formate differed from that of iodide and nitrate. The kosmotropic anions showed a moderate decrease in retention with %ACN up to 80–85%, followed by an increase in retention. This U-shaped retention behavior is similar to that observed for other HILIC phases [20,58,59] indicating that these kosmotropic anions follow a HILIC partitioning at high %ACN. On the other hand, the chaotropic anions showed a dramatic decrease of retention with increasing %ACN, consistent with observations of ion exchangers in mixed aqueous/organic mobile phases [60]. As the amount of ACN in the mobile phase increases, the latex polymer swells. This leads to a decrease in the number of ion exchange sites per volume, and hence lower retention [60]. Other latex coated columns including latices B and C showed comparable behavior to the latex A coated column as shown in Figs. S5 and S6.

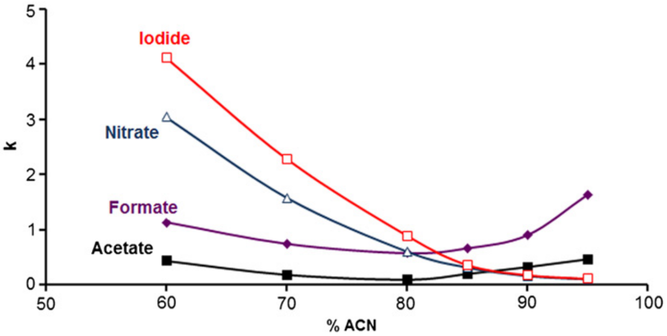

Fig. 7. Effect of %ACN on the retention of anions on the latex A coated Chromolith silica monolith. Conditions: column, latex A coated silica monolith (100 × 4.6 mm ID); flow rate, 1.0 mL/min; eluent, 15 mM TEA-methylphosphonic acid, pH 4.0, in 60–95%ACN; analytes, 0.02–14.7 mM acetate, formate, nitrate and iodide in 65% ACN; UV detection at 210 nm with a 10 μL loop injection. Lines are guides to the eye.

4. Conclusions

The latex nanoparticles provided the silica surface with an 8–10 folds more rich water layer than bare silica phases, leading to greater retention. The AS9-SC (latex A) coated monolith provided the highest ion exchange capacity among the tested latex coated columns. The low degree of cross-linking of the AS12A (latex B) and DNApac (latex C) may account for the lower capacity. Fast separation of kosmotropic and chaotropic anions was achieved on these latex coated monoliths due to the high permeability of the monolith and mixed mode retention mechanism. Latex coated monoliths provided weak anion exchange properties in contrast to the expected strong anion exchange properties. Chromolith and Onyx bare silica monoliths provided very similar selectivity when coated with the same AS9-SC (latex A) nanoparticles. The retention of anions on latex coated silica monoliths is mixed mode in nature. Chaotropic anions are predominantly retained via ion exchange; while kosmotropic anions are predominantly retained via HILIC partitioning.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2012.07.069.

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