Retention of Ionized Solutes in Reversed-Phase High Performance Liquid Chromatography

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RETENTION OF IONIZED SOLUTES IN
REVERSED-PHASE HIGH PERFORMANCE
LIQUID CHROMATOGRAPHY

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ABSTRACT

The mechanism of retention in reversed-phase high performance
liquid chromatography is affected by both solute-eluent interac-
tions and the nature of the stationary phase. The hydrophobic ex-
pulsion of ionized solutes plays a major role in affecting solute
behavior in the water-rich range of hydroorganic eluents. In the
water-lean range of eluent composition, there is little hydropho-
bic expulsion, and specific interactions between the solute and
surface can be observed. The nature of the surface affects the
retention of a variety of ionized species, both large cations and
anions. Octadecylsilane (ODS) bonded phases can exhibit two dif-
ferent binding sites: one exhibiting a weak interaction and the
second a strong specific interaction with a solute. Styrenedi-
vinylbenzene polymeric surfaces exhibit the potential for weak
dispersion interactions, and in addition pi-bonding interactions
with a solute. A variety of solutes have been injected in a water:
methanol eluent system in order to assess solute-surface effects
on reversed-phase supports.

INTRODUCTION

The mechanism of retention in reversed-phase high performance

liquid chromatography has been the subject of controversy. It has

been variously proposed that partitioning of sample molecules oc-

curs into a "liquid" phase defined by the surface and associated

molecules, or that sample molecules adsorb onto the surface (1).

Whatever the nature of the solute-stationary phase phenomena, the

mobile phase affects the extent of interaction of solute with the
stationary phase, either by modifying the surface (2-7) or by in-
teracting directly with the solute in the mobile phase.

Solute-mobile phase interactions can be formulated in terms
of the total energy of interaction of a solute molecule with the
molecules of solvent in the mobile phase. The total energy re-
flects the sum of all possible interactions which include disper-
sion interactions and specific interactions (electrostatic dipole
interactions, electron donor-acceptor interactions and bond for-
mation)(8). These solute-solvent interactions promote the affinity
of the solute for the mobile phase. Countervailing the attractive
interactions is the solvophobic affect (9,10) or hydrophobic ef-
fect when water is the solvent. Water exhibits the strongest
manifestation of this solvophobic effect, and so hydrophobic ef-
fects predominate in the binary eluent systems commonly employed
in reversed-phase HPLC; namely hydroorganic mixtures of water and
methanol, or water and acetonitrile. The hydrophobic expulsion is
generally attenuated by increasing the concentration of organic
modifier. Eventually at high levels of added modifier, e.g. (80:
20) methanol:H_2O for the alkylsulfonates (11), a water-lean eluent
composition is attained and hydrophobic effects are attenuated.
The capacity factor, k is then independent of the number of methy-
lenes in the alkyl chain (11). The eluent composition at which
solvophobic effects are attenuated is dependent on the nature of
the solute, but in general solvophobic effects are negligible at
90% methanol in the mobile phase.

In addition to solute-mobile phase interactions, solute-
stationary phase interactions can play a major role in determining
the extent of retention of a solute in reversed-phase chromato-
graphy. Solvent-surface interactions have been observed on the
surface of C_{18} columns (2-7). Solute-surface interactions are
similar. The interactions may be due to the solute adhering to
the C_{18} group bonded to the silica support (10). In this case,
one can envision a competitive effect in which organic solvent

molecules solvating the n-alkyl bonded chains in the stationary
phase are displaced by solutes of greater hydrophobicity (6).
Alternatively, the solute can interact with adsorbed organic mod-
ifier (3,6). Finally, the nonaqueous modifier in the mobile phase
may be extracted into the bonded phase, and solutes can then par-
tition between the mobile phase and the modified bonded phase (2).
Apart from the solute-hydrocarbonaceous phase interactions, silan-
ophilic interactions (12-14) can be operative causing tailing of
chromatographic peaks and the enhancement of retention of "non-
retained" species at extremely low levels of water. Obviously the
nature of solute-stationary phase phenomena in reversed-phase
chromatography is complex and subject to controversy.

In order to diminish the hydrophobic effects of the mobile
phase in reversed-phase chromatography, experiments were performed
using a water-lean eluent system, (90:10) methanol:H_2O. Solvo-
phobic expulsion of most species is negligible at this eluent com-
position, so that the driving force for retention will not be the
effect of solvent in forcing the solute to the hydrocarbonaceous
surface, but rather potential favorable interactions of the solute
with the stationary phase. In this way solute-stationary phase
interactions can be evaluated without having hydrophobic inter-
actions override the other operative interactions.

In order to assess the role of the stationary phase, two
distinct stationary phases were evaluated: (1) an octadecylsilane
bonded support and (2) a styrenedivinylbenzene co-polymer adsor-
bent. The phases differ significantly in their surface character-
istics.

Finally, quaternary ammonium salts and organic acids were
used as solute ions. These species are most affected by the na-
ture of the support since they can adsorb onto a surface (15). It
is assumed that sample molecules adsorb onto the surface rather
than partition into a liquid phase. An electrical double-layer
model can then be used to describe the retention behavior of ion-
ized solutes in reversed-phase HPLC. Ions which have no primary

solvation layer or are able to lose it on the side facing the ad-
sorbent surface can displace some of the adsorbed solvent molecules
and contact the surface. This contact adsorption is largely gov-
erned by the properties of the given ion. An array of contact ad-
sorbed solvent molecules and solute ions is known as an inner
layer, and the loci of the centers of the contact-adsorbed ions
defines the inner Helmholtz plane (IHP). Solvated ions which can-
not contact adsorb approach the surface up to a distance known as
the outer Helmholtz plane (OHP). These ions are non-specifically
adsorbed since the electrostatic interactions are essentially in-
dependent of the chemical properties of the ions. Counter ions
associated with the contact-adsorbed ions are found in the diffuse
layer outside the OHP. This model is derived from the Stern-Gouy-
Chapman theory of the electrical double layer, and has been used
for describing the adsorption of aromatic ammonium compounds on
Amberlite XAD-2 (16). A similar model was invoked by Bidlingmeyer
et.al. (10) and R. S. Deelder and J. H. M. Van Der Berg (17).

EXPERIMENTAL

The chromatographic system employed in the retention studies
of the anions on the octadecylsilane bonded phase consisted of a
Waters system including an M6000 pump, a U6K injector, a Model 450
variable wavelength detector, a 720 system controller, and a 730
data module.

The chromatographic system used in all the remaining studies
was a Tracor, Model 985 solvent delivery system (Tracor Instru-
ments, Austin, TX, U.S.A.) with a model 7125 automatic syringe
loading sample injector (Rheodyne, Berkeley, CA, U.S.A.) with a
20 μL sampling loop, a model 970A variable wavelength detector
(Tracor), a model 951 HPLC pump (Tracor), and a model 3390A re-
porting integrator (Hewlett-Packard, Avondale, PA, U.S.A.). The
octadecylsilica bonded column was a Partisil 5-ODS (Whatman, Inc.,
Clifton, NJ, U.S.A.), while the styrenedivinylbenzene copolymer
column was a PRP-1 (Hamilton Company, Reno, NV, U.S.A.).

Methanol (MCB Manufacturing, Cincinnati, OH, U.S.A.) was dis-
tilled, then aspirated through 0.50 μM Millipore filters. Filters
were purchased from Rainin, Woburn, MA. Water was distilled and
then aspirated through 0.50 μM cellulose acetate filters. The
mobile phases were degassed using helium prior to use. The mobile
phase flow rate was maintained at 1.00 mL/min.

Tetraalkylammonium salts, including tetramethylammonium
bromide, TMAB, tetraethylammonium bromide, TEAB, tetrabutylammonium
bromide, TBAB, tetraheptylammonium bromide, THAB, and hexadecyl-
trimethylammonium bromide (cetrimide), CTAB, were all purchased
from Eastman, Rochester, NY.

Acids and salts used in this study include: 2-naphthalene-
sulfonic acid, sodium salt (NAS) and picric acid (PA) purchased
from Eastman; toluenesulfonic acid (TS), sodium nitrate ($NaNO_3$)
and potassium nitrate (KNO_3) purchased from Fisher, Fair Lawn, NJ;
dichloroacetic acid (DCA) purchased from Aldrich.

All solutes were prepared as concentrated solutions and then
diluted to the desired mobile phase composition. All samples were
dissolved in the mobile phase. The retention volume of 10^{-4}M
$NaNO_3$ or 10^{-4}M toluenesulfonic acid was used as a measure of the
dead volume in all systems.

RESULTS AND DISCUSSION

Retention of Anions on ODS

A variety of completely dissociated aromatic acids and sodium
salts have been injected into a (25:75) methanol:H_2O eluent system.
In unbuffered systems, as the nanomoles of solute increase, there
is an increase in the capacity factor k_e of the solute. See
Figure 1. The capacity factor k_e is equal to V_R-V_0 / V_0 where V_R
is the eluting volume and V_0 is the void volume in the presence of
pore exclusion phenomena (18). The terminology is analogous to
that used in exclusion chromatography. V_M, the void volume in
general liquid chromatography is the sum of two factors, $V_M = V_e +
\phi V_i$ where V_e is the interparticle void volume and ϕV_i is the intra-

particle fluid volume explored by the solute. In the extreme case
when $\phi = 0$, the solute does not penetrate the pores and the void
volume, V_M is equal to the interparticle void volume, V_e. When
$\phi = 1$, the solute penetrates all the pores and the void volume
equals the sum of the interparticle and intraparticle void volumes.
The value of ϕ should be constant in order to evaluate the capacity
factor k which equals $(V_R - V_M) / V_M$. Using ionized solutes in un-
buffered systems, the pore-penetrating ability of the solute varies
with the ionic strength or amount of solute. The value of ϕ
changes, thus changing the value of V_M. Since V_M is not constant
the capacity factor k is difficult to evaluate. It is inappropri-
ate therefore to correlate an increase in k with an increase in
the chromatographic retention of ionized solutes in unbuffered sys-
tems. For this reason, we have defined the expression, $(V_R - V_0) /$
V_0 as the capacity factor k_e. Berendsen et.a. have considered the
value of V_0 to equal V_R (19); however, it would be difficult to
prove that at low concentrations of electrolyte ($<10^{-3}$M) there is
no pore penetration at all ($\phi = 0$).

The effects in Figure 1 have been observed with ionized
solutes on C_{18} columns (19,20), and have been explained in terms
of a Donnan exclusion phenomena. On the basis of studies with
reversed-phase supports, a salt is excluded from the pores of a
packing at low electrolyte concentrations, presumably because of
electrical charges on the phase support (19). Silica gel is known
to have a negatively-charged surface at pH values above 2 because
of the ionization of silanols (21). Alternatively, the adsorption
of the first few molecules on the surface may impede any addition-
al adsorption, and the existance of a pre-charged surface is not
necessary in order for a Donnan expulsion phenomenon to be ob-
served. This Donnan exclusion effect can be eliminated by adding
a supporting electrolyte.

Counteracting the surface charge expulsion, the hydrophobic
effect forces the solute to the surface. The expulsion of the
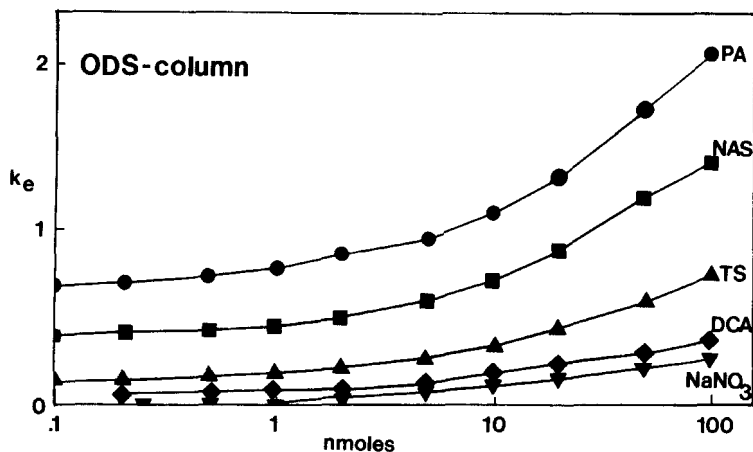anions from the eluent is based on molecular size such that the

Figure 1. Retention versus Amount Injected for a Series of Anions.
Mobile phase (25:75) methanol:water. Column: Whatman ODS-5.
Samples: PA = picric acid; NAS = naphthalene sulfonic acid; TS =
toluenesulfonic acid; DCA = dichloroacetic acid; NaNO$_3$. Detection
wavelengths: PA = 350 nm, NAS = 228 nm, TS = 203 nm, DCA = 203 nm,
NO$_3$ = 203 nm.

retention volume increases with number of carbon atoms (9). For

the following ionized species: DCA, TS, NAS and PA, the capacity

factor k_e increases with the increasing size of the anion. See

Figure 1.

As the organic modifier concentration increases, k_e diminishes

and approaches that of the "unretained" NaNO$_3$ at a given concentra-

tion of solute. Eventually any hydrophobic effects are attenuated

and the molecular size does not affect the retention. There is no

hydrophobic expulsion which differentiates the anions from each

other, so that the concentration effects are identical. Figure 2

shows the similarity between the elution behavior of picric acid

and potassium nitrate. This behavior is observed for all the com-

pletely dissociated aromatic acids and salts investigated at (90:

10) methanol:H$_2$0.

In order to substantiate that the variation in sorption of

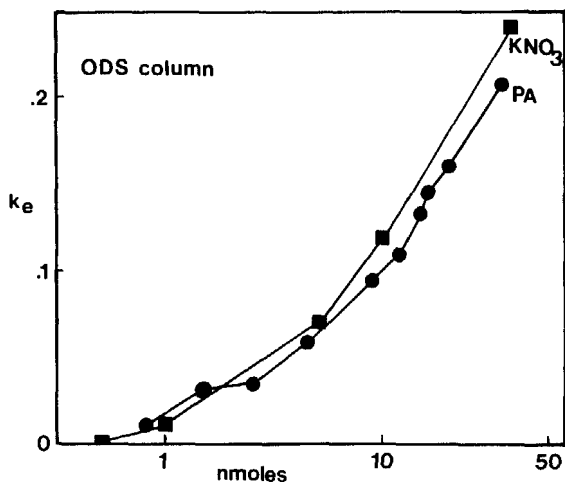solute is indeed due to an electrostatic expulsion of the eluite

Figure 2. Retention versus Amount Injected. Mobile phase:
(90:10) methanol:water. Column: Whatman ODS-5. Samples: PA =
picric acid; KNO_3. Detection wavelengths same as Figure 1.

from the surface, $NaNO_3$ was added to the mobile phase. With in-
creased electrolyte concentration in the mobile phase, the ion ex-
clusion effect should be diminished, and the pores should then be-
come accessible to the solute (19). Maintaining the solute con-
centration constant, as the concentration of electrolyte in the
mobile phase increases, the retention of picric acid also in-
creases. The enhancement of retention on addition of electrolyte
to the mobile phase mimicks that of the behavior observed on
varying the amount of solute injected in the absence of electro-
lyte. "It is immaterial whether the enhanced ionic strength of
the mobile phase results from the injected salt itself or from
an electrolyte added to the mobile phase" (19). This would seem
to further support Donnan exclusion as the basis for the minimal
retention of solutes at low ionic strength. A simple "salting out"
effect cannot be invoked to explain the enhancement of retention
of solutes at high ionic strength because this effect would not be
paramount at ionic strengths below 0.1 M. (9).

Retention of Cations on ODS

Octadecylsilica bonded phases exhibit ion exchange properties
due to the acidity of the silanols on the surface (21). Cations
can exchange with the protons bound to residual silanol groups (21)
and thus the nature of the support can be modified by adding elec-
trolyte. Presumably, the surface will have a heterogeneous mixture
of SiO^-H^+ and SiO^-M^+ (M^+ is the cation), with the exact surface
concentrations determined by the concentration of electrolyte in
the eluent. This cation exchange behavior has implications for the
mechanism of retention of quaternary ammonium salts. These species
have the possibility for interacting with the surface at two dis-
tinct binding sites. The lipophilic quaternary ammonium ion can
be adsorbed onto the nonpolar surface (22). Alternatively, the
quaternary ammonium ions might exchange against protons and any ca-
tions contributed by added electrolyte. These two distinct binding
mechanisms were postulated by van der Houwen et.al. (23). Further-
more, the work of Melin et.al. (24) and Eksborg and Ekquist (25)
supports the idea of two binding sites in alkyl-silica bonded phases.

In order to assess the mechanism of retention of cations on
octadecylsilica bonded phases, a series of quaternary ammonium ions
were injected into a water-lean eluent system (90:10) methanol:H_2O.
For the small quaternary ammonium salts, TMAB and TEAB, there is a
small increase in the retention with amount injected. See Figure 3.
This can be explained in terms of Donnan expulsion. The two species
are not differentiated on the basis of molecular size. Apparently,
the difference in hydrophobicity of the two species is insufficient
to produce any distinction in their chromatographic properties. As
the size of the quaternary ammonium ion increases, the capacity
factor k_e increases. Solvophobic effects appear to differentiate
the large quaternary ammonium ions on the basis of size (k_e of
THAB > TBAB > TEAB). This is not entirely unexpected since large
quaternary ammonium ions are extremely solvophobic, and they might
be expelled even in pure methanol as the eluent. It is assumed
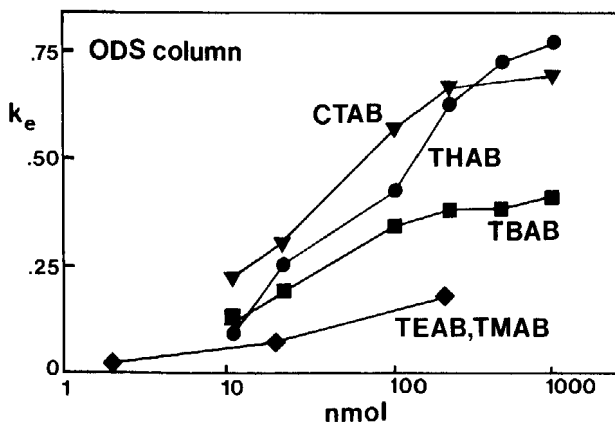with the quaternary ammonium salts that the prominant basis of

Figure 3.   Retention versus Amount Injected for a Series of
Quaternary Ammonium Salts.   Mobile phase: (90:10) methanol:water.
Column: Whatman ODS-5.   Samples: CTAB = hexadecyltrimethyl-
ammonium bromide, THAB = tetraheptylammonium bromide, TBAB =
tetrabutylammonium bromide, TEAB = tetraethylammonium bromide,
TMAB = tetramethylammonium bromide.   Detection wavelength = 203 nm.

differential retention on the surface is due to the hydrophobic
expulsion of the salt, and that the secondary interactions between
solute and surface are not the primary basis for retention.   Both
binding sites on ODS-bonded phases are presumably involved in the
retention of quaternary ammonium salts (23).

Cetrimide,   CTAB, exhibits anomolous retention behavior.   See
Figure 3.   Whereas all the spherically-symmetric surfactants show
a regular increase in the k_e as the nanomoles of solute injected
increase, CTAB exhibits a levelling effect at high solute concen-
trations.   Presumably, this can be ascribed to the fact that CTAB
can more easily coat the surface by aligning its extended tail a-
long the C_{18} hydrocarbon moiety on the surface.   This would then
cause a surface saturation to occur at relatively low concentra-
tions of solute.   THAB on the other hand is a large spherical
hydrophobic species which has a positive charge located 7 carbon
atoms away from the end of the heptyl moiety.   It cannot contact
adsorb as well on the surface because of the limited number of

carbon atoms which can align with the surface. It would therefore
not saturate the surface as completely as a linear long-chain
quaternary ammonium salt at a similar concentration.

Cations on PRP-1

Figure 4 depicts the retention behavior of quaternary ammonium
salts on a PRP-1 column. The PRP-1 column packing consists of
rigid spherical 10 μm particles which are a copolymer of styrene
and divinylbenzene. The packing material is an adsorbent which
can be used in the reversed-phase or normal phase modes. The sur-
face has no Bronsted acid character, and therefore there is no
possibility for bonding quaternary ammonium ions to cation-exchange
sites. The quaternary ammonium ions must therefore be adsorbed on
the surface. Recently, Cantwell and Puon (16) used a model derived
from the Stern-Gouy-Chapman double-layer theory for describing the
adsorption of aromatic ammonium compounds on Amberlite XAD-2. The
surface of PRP-1 is chemically similar to that of Amberlite XAD-2.
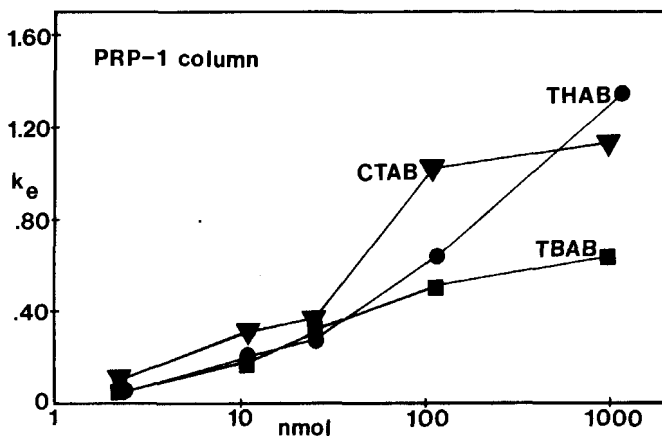Therefore, the quaternary ammonium ions would form a primary layer

Figure 4. Retention versus Amount Injected for a Series of
Quaternary Ammonium Salts. Mobile phase: (90:10) methanol:water.
Column: PRP-1. Samples and detection wavelengths same as Figure 3.

adsorbed to the surface while the bromide counterions would then
be located in the diffuse layer.

In order to explain the chromatographic retention of these
ionized solutes, a Donnan exclusion effect can be invoked. As the
concentration of quaternary ammonium salt increases in the solute
plug, the local ionic strength increases, and the Donnan exclusion
is diminished. This explanation would correlate with that given
for the ODS bonded phases. Alternatively, a non-specific salting
out effect has also been postulated (26,27).

As far as the differing retention behavior, See Figure 4,
this can be explained in terms of hydrophobic and steric effects.
THAB is expelled more than TBAB by the eluent and therefore the
retention of THAB is greater than that of TBAB. CTAB again exhib-
its distinctive behavior differing from that of the spherically-
symmetric surfactants because of its ability to maximize dispersion
interactions with the hydrophobic surface. CTAB can align its
hexadecyl moiety with the surface and position its positive charge
away from the surface. THAB has its positive charge located 7 car-
bon atoms from the end of the heptyl moiety, and it is therefore
more difficult to isolate the charge from the surface. Contact
adsorption is easier for CTAB, and therefore surface saturation
would occur at lower concentrations of added solute.

The most important fact to be gleaned from the experimental
data is that the nature of the surface (for hydrophobic surfaces)
does not appear to have a dominant effect on the retention of large
cations. Even though ODS bonded-phases exhibit a potential ion-
exchange interaction, it appears that hydrophobic effects dominate
the retention behavior of quaternary ammonium salts on ODS and poly-
styrenedivinylbenzene copolymer surfaces.

Anions on PRP-1

Figure 5 shows the chromatographic behavior of a series of
ionized salts and completely dissociated acids on PRP-1. The
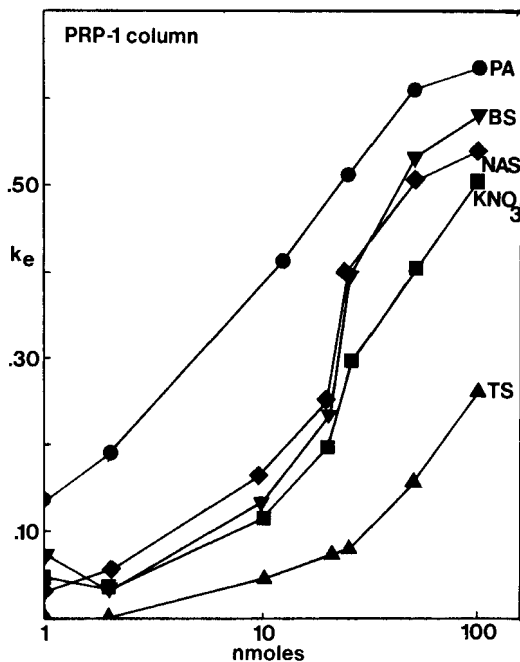anions can contact adsorb on to the surface. Pietrzyk and Chu

Figure 5.   Retention versus Amount Injected for a Series of Anions.
Mobile phase (90:10) methanol:water.   Column: PRP-1.   Samples and
detection wavelengths same as Figure 1.

(27) envision the sorption of neutral aromatic acids as close to
a flat-wise orientation.   If the organic acid is in its salt
form, the charged group disrupts the binding, and the orientation
of the solute is probably more end-on with the charged site away
from the XAD surface (27).   Whatever the sorption orientation on
the surface, a charged layer is formed and Donnan expulsion can
occur in unbuffered eluents.   This explains the increase in k_e
with amount of injected solute.

    Figure 5 demonstrates a variation in the relative retenion
of anions on the PRP-1 surface.   This contrasts sharply with the
behavior observed on the ODS surface.   At (90:10) methanol:water,
none of the anions injected are differentiated on the ODS surface.

All of the anions exhibit identical k_e values at all concentra-
tions. Hydrophobic effects are not operative for these homologues
at an eluent composition of (90:10) methanol:water. However, on
the PRP-1 surface, the nature of the anion affects the k_e at a
given concentration of solute. Since the effects cannot be at-
tributed to the eluent, solute-surface interactions on polystyrene-
divinylbenzene are implicated. The relative retention order for
the species is TS < KNO_3 < BS, NAS < PA. This is not the expected
order on the basis of size, but then again since solvophobic ef-
fects are not operative, this is not surprising. The explanation
for this ordering lies in the nature of the surface. Styrenedi-
vinylbenzene polymeric surfaces exhibit the potential for weak
dispersion interactions and in addition pi-bonding interactions
with the solute. Benzene sulfonic acid (BS) and the sodium salt
of naphthalene sulfonate (NAS) can align themselves with benzyl
groups on the surface and maximize the pi-bonding interactions.
These two aromatic sulfonates can orient themselves in such a way
as to position the negative charge away from the backbone of the
copolymer. The nitrate anion can undergo charge transfer complex-
ation with the aromatic moieties of the copolymer and exhibit re-
tention behavior analogous to that of the aromatic sulfonates.
Toluene sulfonic acid (TS) has lower k_e values at all concentra-
tions of solute. See Figure 5. This is attributed to the methyl
substituent on the benzene sulfonic acid. The methyl group pre-
sumably limits the distance of closest approach between the sur-
face and the aromatic ring. Finally, picric acid (PA) exhibits
the highest k_e values at all solute concentrations. Picric acid
is a relatively strong Lewis acid and the charge density of the
aromatic ring is low. Lewis acid-base behavior is expected then
between the benzyl groups of the copolymer and the aromatic tri-
nitrophenol. Picric acid is known to form adducts with aromatic
species, and therefore high retentivity is expected.

CONCLUSIONS

In water-lean eluents, where hydrophobic effects are diminished, surface effects can be observed. Anions interact with polystyrenedivinylbenzene surfaces on the basis of specific interactions. Anions do not exhibit any specific interactions with octadecylsilane bonded phases, unless of course the amount of water in the eluent is so low that silanols are not completely solvated. Large quaternary ammonium ions interact with both ODS and polystyrenedivinylbenzene surfaces in a similar manner. Solvophobic effects appear to dominate the retention behavior. Perhaps if an eluent system did not exhibit solvophobic expulsion of large quaternary ammonium ions, specific interactions between the ammonium ion and the surface could be observed; however, this has not been confirmed experimentally. Finally, both quaternary ammonium ions and aromatic acids and salts demonstrate an enhancement of retention as the amount of solute increases. This can be ascribed to Donnan expulsion of the solute at low ionic strength. The first molecules which adsorb on the surface, prevent the additional sorption of other molecules. If the solute concentration is then increased, the increased ionic strength of the solute plus swamps the Donnan expulsion effect and more solute molecules can then sorb to the surface.

ACKNOWLEDGEMENTS

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