How Theory and Practice Meet in Ion Pair Chromatography

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Outline of the presentation

- What is Ion Pair Chromatography (IPC)?
- Introduction to the theoretical concepts.
- A simplified, but practical, model for IPC.
- How to use the simple model in practical work.
- Comparison between the simplified model and experiments.
- Conclusions.
What is Ion Pair Chromatography?

• Ion Pair Chromatography (IPC) is used to separate ionic analytes on a reversed phase column.

• An Ion Pair reagent is added to modulate retention of the ionic analytes.

• Typical IP reagents are: alkylsulphonates, alkylsulphates, tetra-alkylammonium ions etc.
What is the effect of the IP-reagent on retention of analytes of different charge?

No IP-reagent
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No IP-reagent

+ charged IP-reagent
What is the effect of the IP-reagent on retention of analytes of different charge?

- **No IP-reagent**
  - + charged analytes
  - - charged analytes

- **+ charged IP-reagent**
  - + charged analytes
  - - charged analytes

- **- charged IP-reagent**
  - + charged analytes
  - - charged analytes

Retention time
Schematic explanation of the effect of the IP-reagent on retention

When no IP regent is added the distribution is 1:1 for all analytes.
Schematic explanation of the effect of the IP-reagent on retention

The IP-reagent will adsorb to the stationary phase.
Schematic explanation of the effect of the IP-reagent on retention.

The charge of the adsorbed IP-reagent interacts electrostatically with the charge of the analytes.
Experimental parameters in IP-chromatography

• Type of analyte (charge, hydrophobicity, etc).
• Type of IP-reagent (charge, hydrophobicity etc.)
• Concentration of IP-reagent.
• Type of organic modifier in the mobile phase.
• Concentration of organic modifier in the mobile phase.
• Ionic strength and type of ions in the mobile phase.
How theory meet practice in IP-chromatography.

- A theory for IP-chromatography shall quantitatively describe how retention is affected by:
  - The charge of the analyte.
  - The type and concentration of IP-reagent.
  - The type and concentration of organic modifier.
  - The ionic strength.
- The theory shall be physically acceptable.
Charged surface in contact with an electrolyte.

How are ions distributed close to a charged surface?
Charged surface in contact with an electrolyte.

How are ions distributed close to a charged surface?
At equilibrium a minimum in free energy is achieved. A difference, $\Delta \Psi$, in electrostatic potential between the surface and the electrolyte is created.
Thermodynamics of adsorption of the IP-reagent and retention.

\[ \Delta G_{\text{tot}} = \Delta G_{\text{chem}} + zF\Delta \Psi \]
Implications of the electrostatic retention model.

- $\Delta \Psi$ depends only on the concentration of IP-reagent on the surface and is independent of the type.
- $\Delta G_{\text{chem}}$ is more or less independent of the type and concentration of the IP-reagent.
- The retention factor is described by the equation:
  \[ k = k_0 \exp\left(-\frac{zF\Delta \Psi}{RT}\right) \]
  where $k_0$ is the retention factor in absence of the IP-reagent.
Implications of the electrostatic retention model.

• For a given analyte does retention depends on the surface concentration of the IP-reagent only. It is independent of the type of IP-reagent,

• Example: Retention factor of adrenaline as a function of the surface concentration of different anionic IP-reagents.
Experimental data from Bartha et.al, J. Chromatogr. 303 (1984) 29

Fig. 4. Capacity factor ($k$) data for adrenaline vs. stationary phase concentration ($n_A$) of sodium (△) butyl-, (*) hexyl- and (□) octylsulphonate pairing ions measured at constant ionic strength (175 mM Na$^+$) of the phosphate buffer (pH 2.1) mobile phase on an ODS-Hypersil column. See ref. 26 for experimental details.
The Gouy-Chapman Theory

- A physically based theory for a planar charged surface in contact with an electrolyte solution (a solution of the Poisson-Boltzmann eq.).
- The electrolyte is assumed to consist of point charges.
- The surface charges are assumed to be evenly smeared out.
- Describes the surface potential as a function of surface charge density and the ionic strength of the electrolyte.
Outline of a simplified electrostatic theory for IP-chromatography.

- $\Delta \Psi$ depends linearly on the surface concentration of IP-reagent and on the inverse of the ionic strength of the mobile phase.

- Both the IP-reagent and the analyte are fully ionized.

- For a given column does $\Delta G_{\text{chem}}$ for both the IP-reagent and the analyte depend only on the mobile phase composition, i.e. not on $\Delta \Psi$.

- The simplifications are reasonable when the ratio $k_{\text{cIP}}/k_0$ is between 2 - 10
Simplified theory, cont.

• Using approximations of the G-C theory and some algebra the following equation can be obtained:
Simplified theory, cont

- Using approximations of the G-C theory and some algebra the following equation can be obtained:

\[
\ln k_{cA} = \ln k_{0A} + \frac{-Z_A Z_{IP}}{Z_{IP} + 1} \ln((n_0 K_{IP} C_{IP} / \kappa) + \text{const})
\]

Capacity factor at concentration \( C_{IP} \)

Capacity factor at concentration \( C_{IP} = 0 \)

Electrostatic contribution as a function analyte charge, \( Z_A \), IP reagent charge \( Z_{IP} \), its binding constant \( K_{IP} \), the conc. of the IP-reagent \( C_{IP} \) and ionic strength. \( \kappa \) is inversely proportional to \( I^{1/2} \).
Retention as a function of the charge of the IP-reagent and the analyte.

- The simplified theory predicts that the slope of a $\ln k_A$ vs $\ln c_{IP}$ plot is:
  - $\frac{1}{2}$ for monocharged analytes and IP-reagents of opposite charge.
  - $-\frac{1}{2}$ for monocharged analytes and IP-reagents of the same charge.
  - 1 for dicharged analytes and monocharged IP reagents of opposite charge.
Comparison of theoretical and experimental results for the slope of the $\ln k_A$ vs $\ln c_{IP}$, monocharged IP reagent.

When $z_{IP} = +2$ and $z_A = -1$ the theoretical slope is 0.4 and when $z_A = -2$ it is 0.8.


Fig. 6. Capacity factor ($k$) data for singly ($\Delta = 1$-naphthylamine-4-sulphonic acid; $\ast = 6$-naphthol-2-sulphonic acid) and doubly charged sulphonic acids ($\Box = 2$-naphthol-6,8-disulphonic acid; $\Diamond = \text{naphthalene}-2,7$-disulphonic acid) as a function of eluent concentration ($c_A$) of a doubly charged pairing ion, hexamethonium bromide ($z_A = +2$). Data were measured by Petterson and Schill [27] using a phosphate buffer (pH 5.5) at constant ionic strength (0.1 $M$) on a LiChrosorb RP-18 column.
Simplified theory, cont

• Using approximations of the G-C theory and some algebra the following equation can be obtained:

\[ \ln k_{CA} = \ln k_{0A} + \frac{Z_A Z_{IP}}{Z_{IP}^2} \times \ln((n_0 K_{IP} c_{IP} / \kappa) + \text{const}) \]

Retention factor of the analyte.

Depends on the concentration of the organic modifier in the mobile phase.
Retention as a function of organic modifier concentration.

From reversed phase chromatography of non-charged analytes it is known that the retention factor as function of organic modifier concentration follows the following equation:

\[ \ln k = \ln k_0 - S \phi \]

where \( k_0 \) is the retention factor at modifier concentration zero. \( S \) is a constant which is characteristic for the analyte and \( \phi \) is the organic modifier concentration.
Simplified theory, cont.

The approximate equation derived from the simplified theory includes both electrostatic effects and the effect of varying the organic modifier content of the mobile phase.

The equation can therefore be used for predictive purposes.

Example: The combined effect of varying the IP reagent and the organic modifier concentration.

Analyte: Octopamine.
IP reagent: Octylsulfonate
Organic modifier: Methanol
Prediction of retention by the simplified theory.

Two experimental points at two different organic modifier concentrations can be used to predict a large number of experimental results.
Prediction of retention by the simplified theory, cont.

At both modifier concentrations the slope of log k vs log cIP is 1/2.
From the difference in the ordinate values can the value for $S$ be calculated.
It is now easy to predict the log k vs log cIP at a third organic modifier concentration, in this case 40% MeOH.

Prediction of retention by the simplified theory, cont.

- Analogously, from two experimental points, the retention of Np-sulphonate as a function of the octylsulphonate concentration can be predicted for various organic modifier concentrations.

Conclusions: Features of the Electrostatic theory for Ion Pair Chromatography

- The theory and its parameters are physically well defined.
- It encompasses all possible combinations of charge of the IP-reagent and the analyte.
- There is good agreement between theory and experiments.
- A simplified form of the theory is simple to use.
- The simplified form can be used for prediction of capacity factors under many different experimental conditions.
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• To YOU for listening.