OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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APPLICATION NOTE

Hydrophobic Interaction Chromatography: Facts

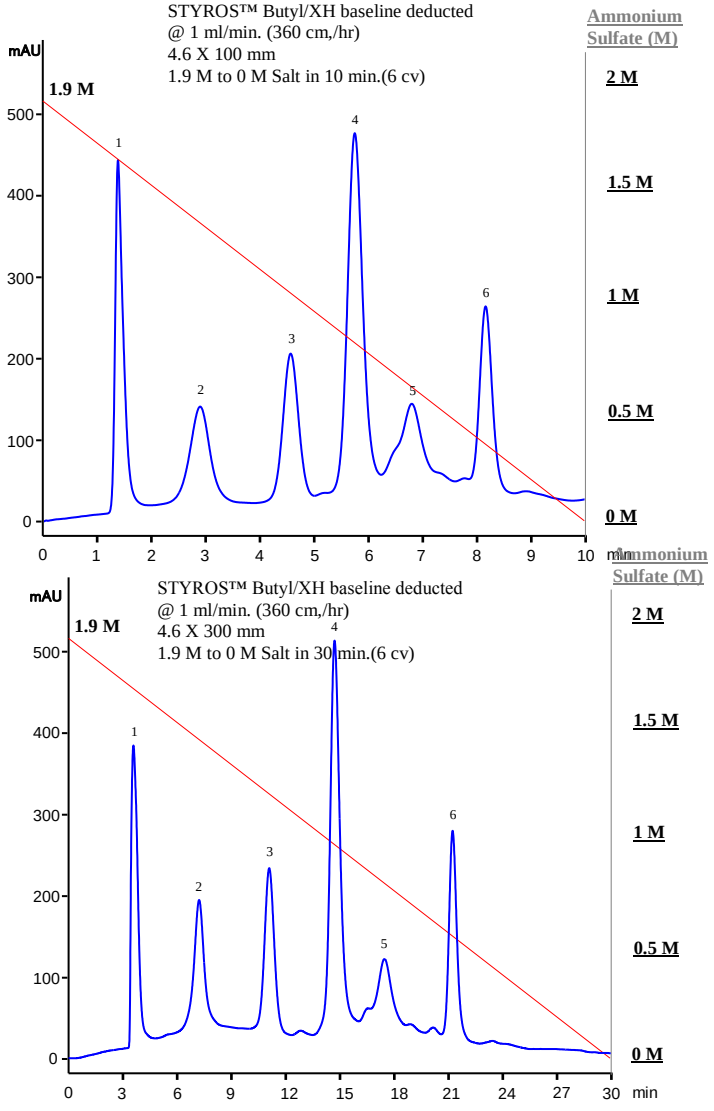
Hydrophobic Interaction Chromatography or HIC is based on the adsorption of biomolecules such as proteins through non-ionic interactions between non-polar regions on the protein's surface and the hydrophobic surface of the stationary phase.
It is usually performed during an elution starting with high salt concentrations.
Reversed phase chromatography or RPC, on the other hand consists of binding the proteins in a polar mobile phase and reducing the polarity of the mobile phase during elution.

The two methods have the following differences.

HIC	RPC
Non denatured proteins	*Denatured proteins*
Adsorption chromatography	*Partition chromatography*
Weaker interaction	*Stronger interaction*
Less hydrophobic ligands	*More hydrophobic ligands*
Elution with reducing salt in water.	*Elution with organic non polar solvents.*
Matrix less substituted	*Matrix more substituted*

One realizes that the denaturation of the biomolecule of interest is to be taken into account as its refolding will impose an insurmountable challenge and would add an extra step to the process.
The ratio of the resolution or the theoretical plates over the back pressure of the column (N/ΔP) is also an important factor to be considered when staging the separation.

The residency time is controlled by the linear speed (expressed in cm/hr) which itself is a function of the internal diameter of the column (ID).
By way of an example the end user would need to compare the data provided by different supplier in a column with an ID of 7.8mm with a column of 4.6mm ID.
Should the data indicate that both columns were run with a volumetric flow rate of 1ml/min one would understand that the linear flow rate of one (7.8mm ID column) was 126 cm/hr as compared with 361 cm/hr with the other (4.6mm ID column).
This is at a speed 2.9 times faster and therefore reduces the process time by a factor of 2.9.
The other factors then come into play.
That is the property of the media; its physical and chemical stability; the life of the column; its leaching and degradation.
Can it support any pH that is needed for the separation; is the back pressure prohibitive? Does it provide the proper resolution for the separation?
In summary, does it add a new tool in my arsenal or is it another product with its limitations?
All these are pertinent questions to ask if we were to promote an improvement in the supply of non-leaching and stable media for the tasks before us.
Here we have provided an example of separation using a mixture of 6 different proteins (Cytochrome c, 2- Myoglobin, 3-Ribonuclease A, 4- Lysozyme, 5- Ovalbumin, 6- α-Chymotrypsinogen A 1 mg/ml each in buffer A) with two columns of different length and similar ID of 4.6 mm.



Despite its longer length and higher resolution the back pressure of the 300 cm column is less than 30 bar during the run with 1.9M salt and 1ml/min volumetric flow.
The proteins were monitored at 214mn for added sensitivity.