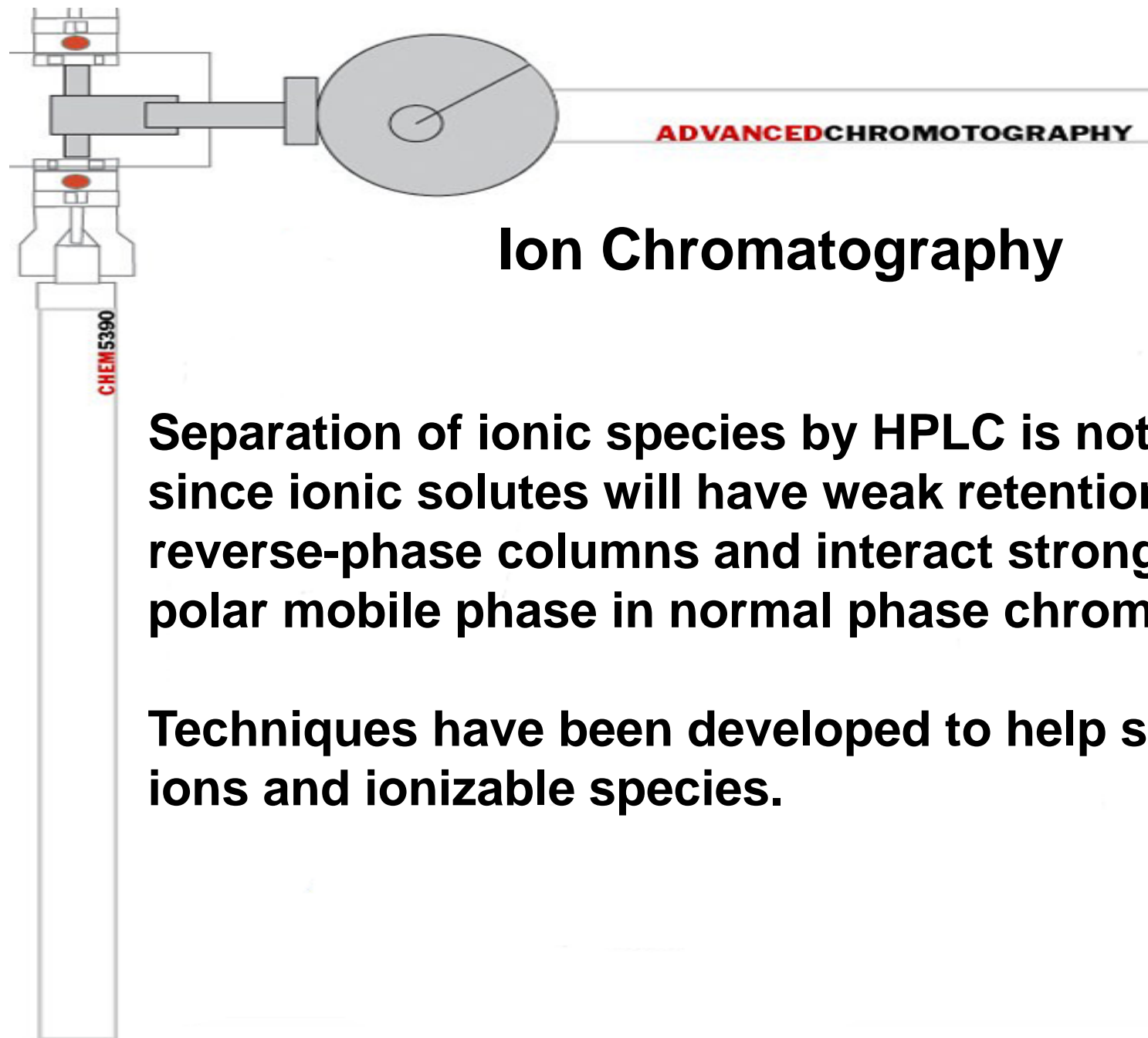


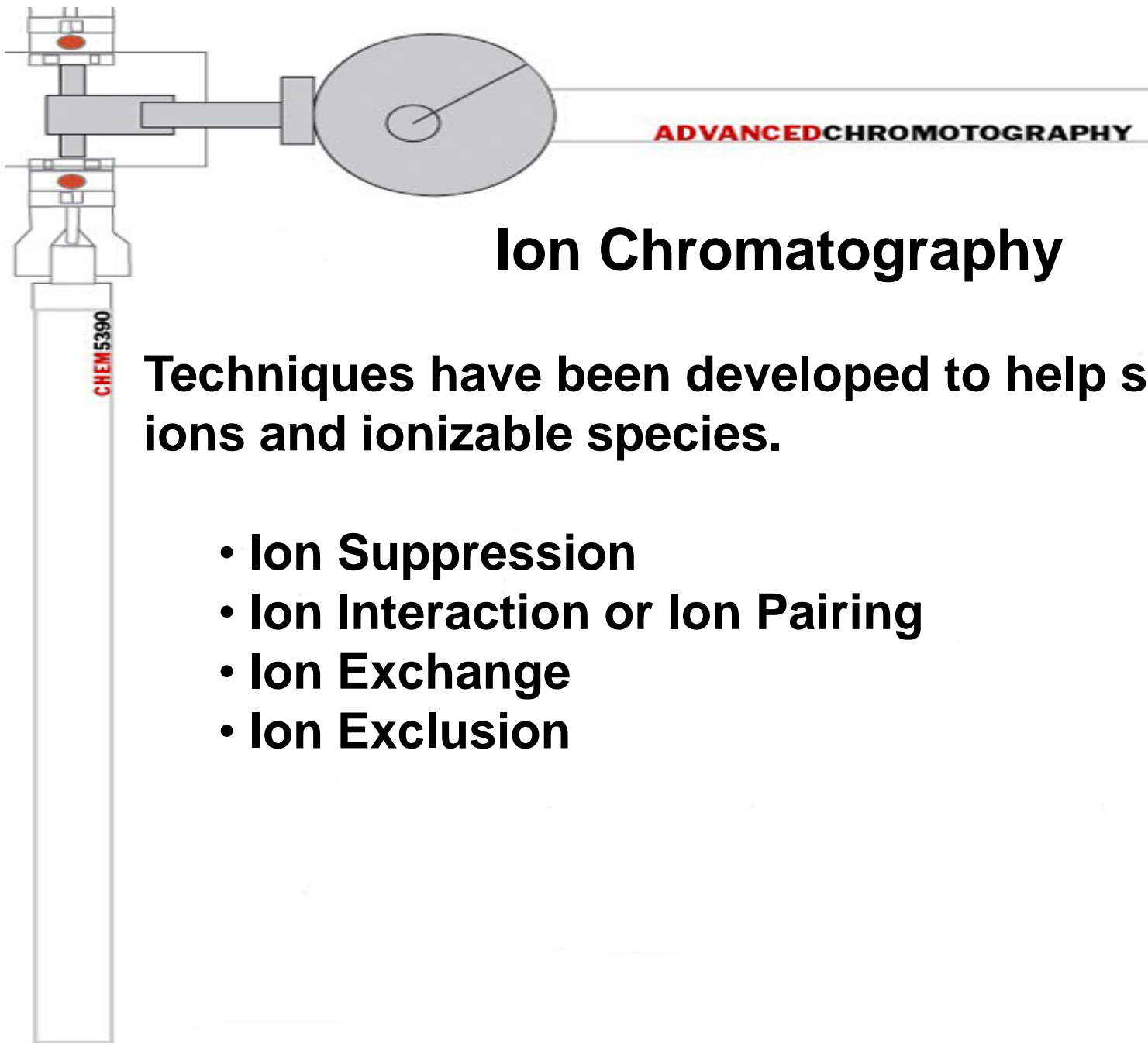
Lecture 18: Ion Chromatography



Ion Chromatography

Separation of ionic species by HPLC is not practical since ionic solutes will have weak retention on reverse-phase columns and interact strongly with the polar mobile phase in normal phase chromatography.

Techniques have been developed to help separate ions and ionizable species.



Ion Chromatography

Techniques have been developed to help separate ions and ionizable species.

- **Ion Suppression**
- **Ion Interaction or Ion Pairing**
- **Ion Exchange**
- **Ion Exclusion**



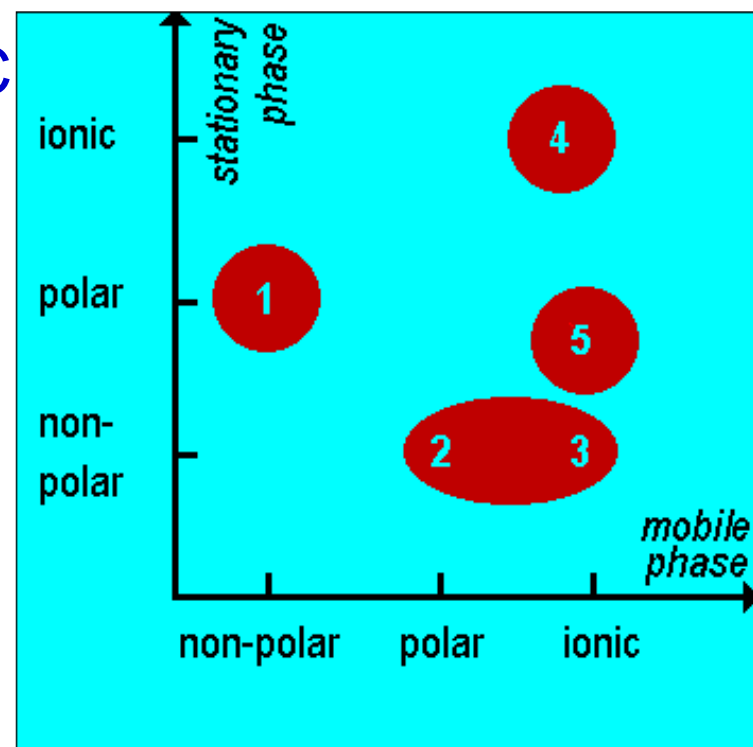
Ion Chromatography

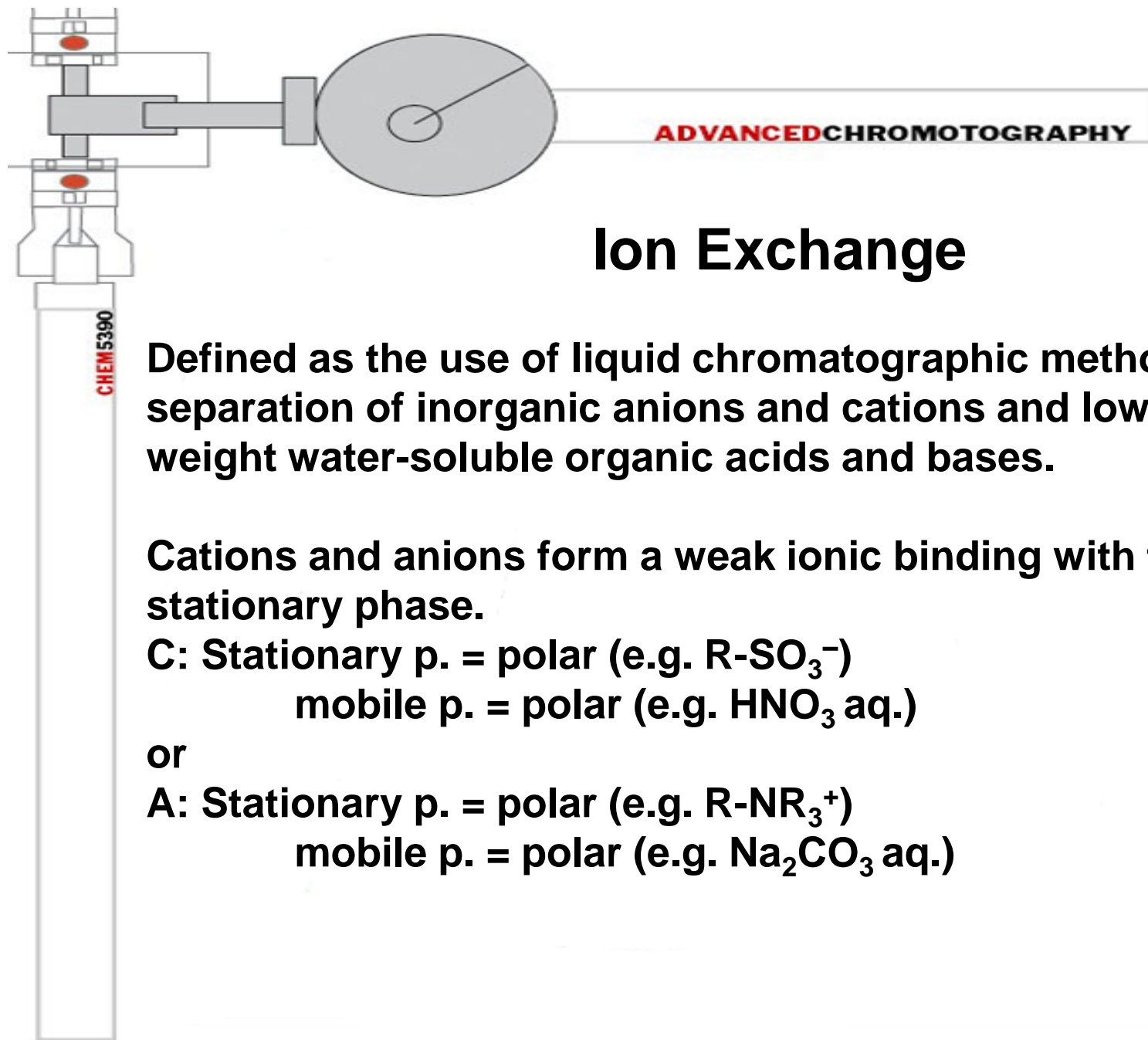
Group 1– traditional TLC + HPLC

1. normal phase chromatography
2. reversed phase chromatography

Group 2– Ion Chromatography

4. ion exchange chromatography
3. ion pair chromatography
5. ion exclusion chromatography





Ion Exchange

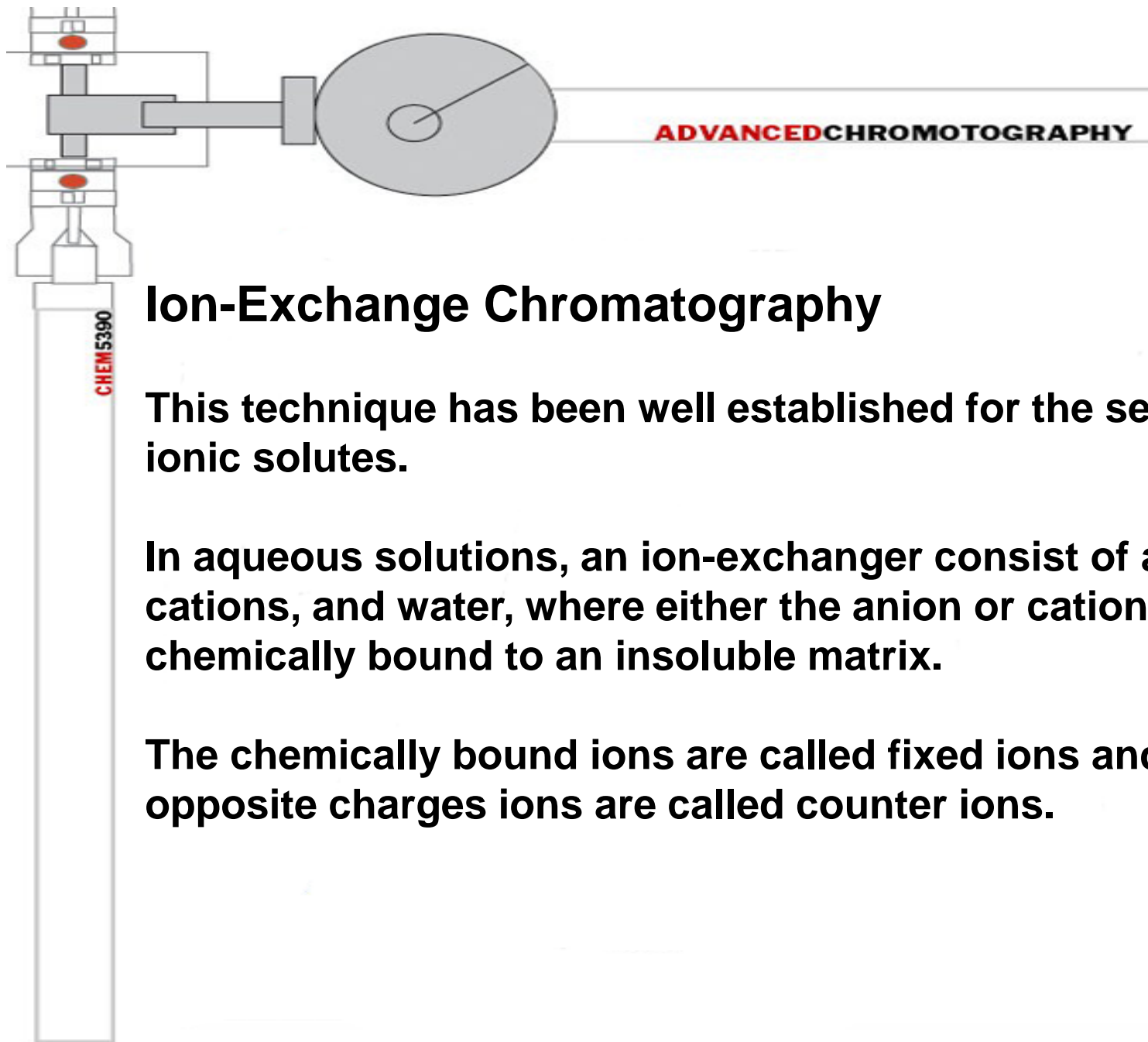
Defined as the use of liquid chromatographic methods for the separation of inorganic anions and cations and low molecular weight water-soluble organic acids and bases.

Cations and anions form a weak ionic binding with the stationary phase.

**C: Stationary p. = polar (e.g. R-SO_3^-)
mobile p. = polar (e.g. HNO_3 aq.)**

or

**A: Stationary p. = polar (e.g. R-NR_3^+)
mobile p. = polar (e.g. Na_2CO_3 aq.)**

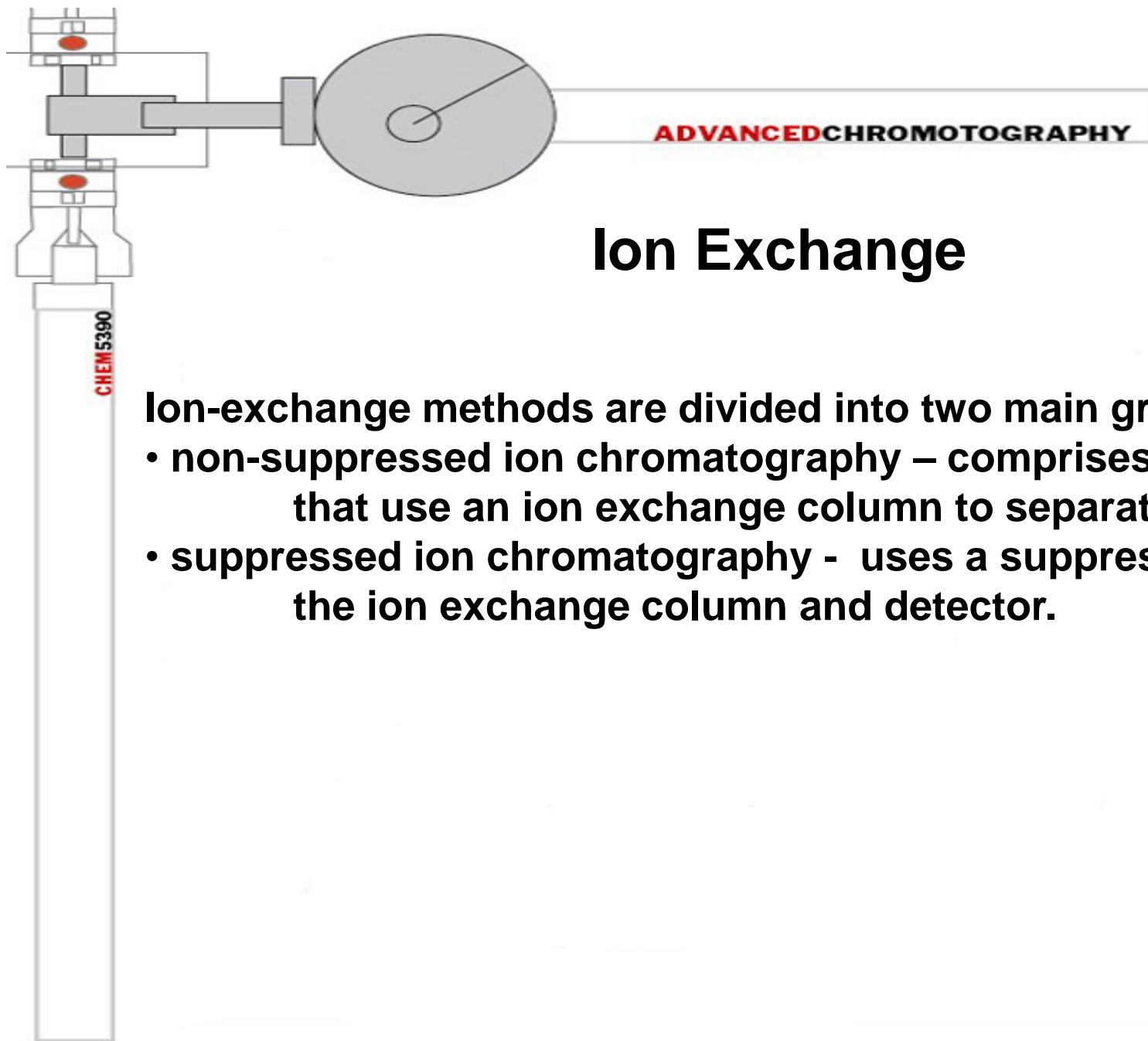


Ion-Exchange Chromatography

This technique has been well established for the separation of ionic solutes.

In aqueous solutions, an ion-exchanger consist of anions, cations, and water, where either the anion or cation are chemically bound to an insoluble matrix.

The chemically bound ions are called fixed ions and the opposite charges ions are called counter ions.

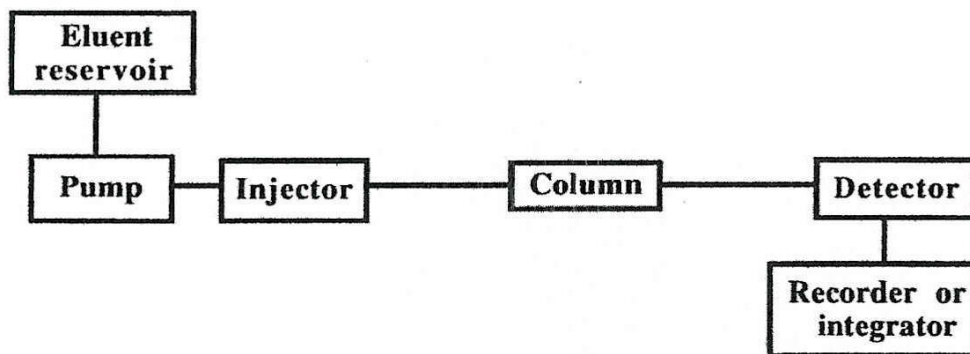


Ion Exchange

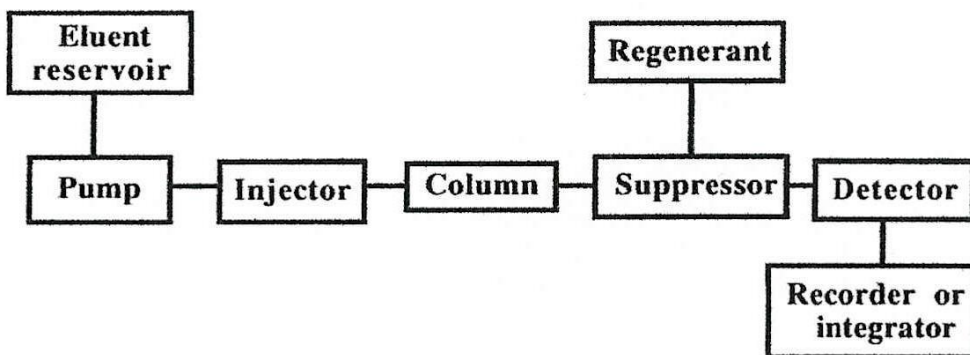
Ion-exchange methods are divided into two main groups

- non-suppressed ion chromatography – comprises all methods that use an ion exchange column to separate mixtures**
- suppressed ion chromatography - uses a suppressor between the ion exchange column and detector.**

Ion Exchange

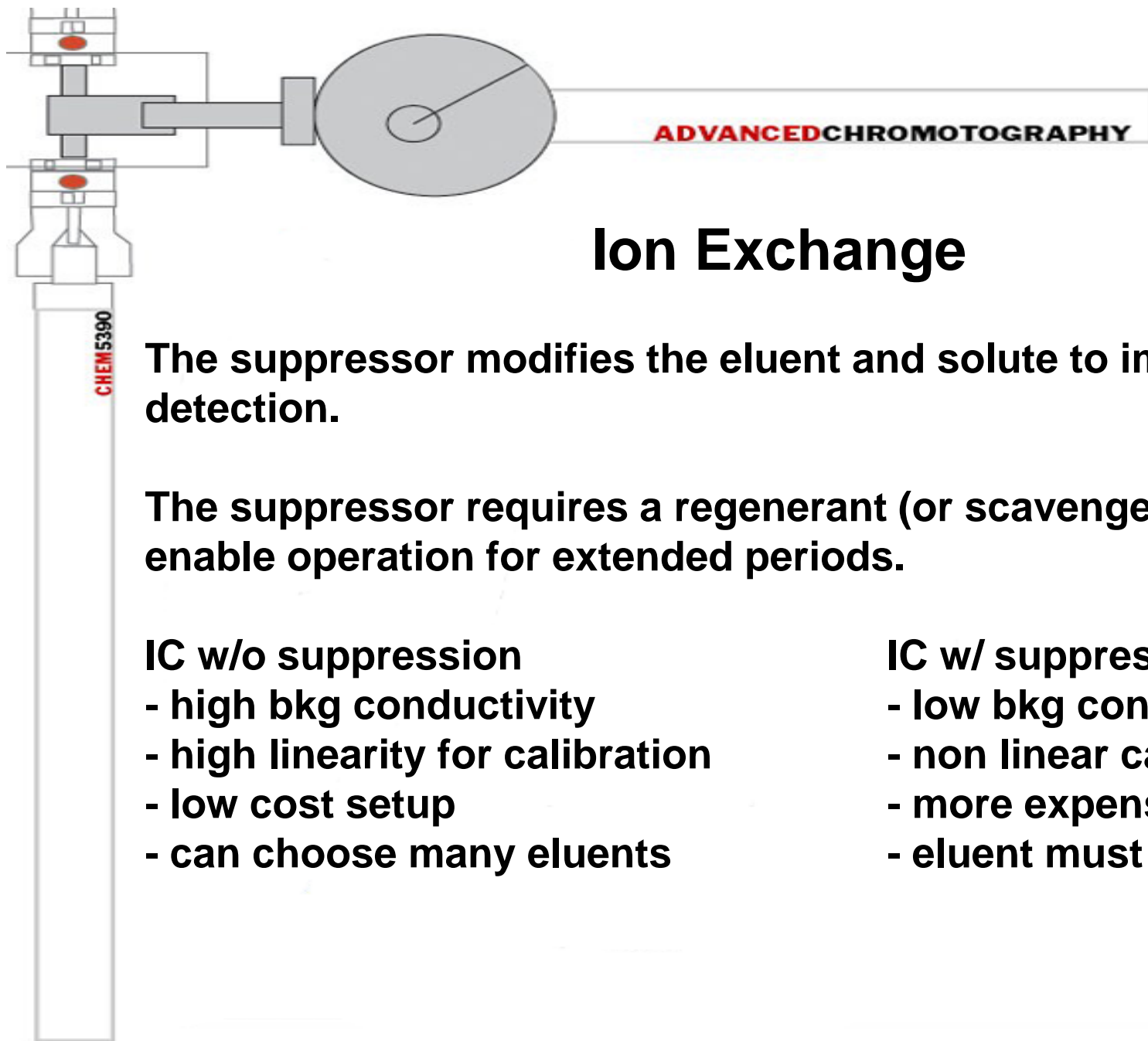


(a)



(b)

Fig. 6.34. Block diagram showing the instrumental components used in (a) nonsuppressed and (b) suppressed IC.



Ion Exchange

The suppressor modifies the eluent and solute to improve detection.

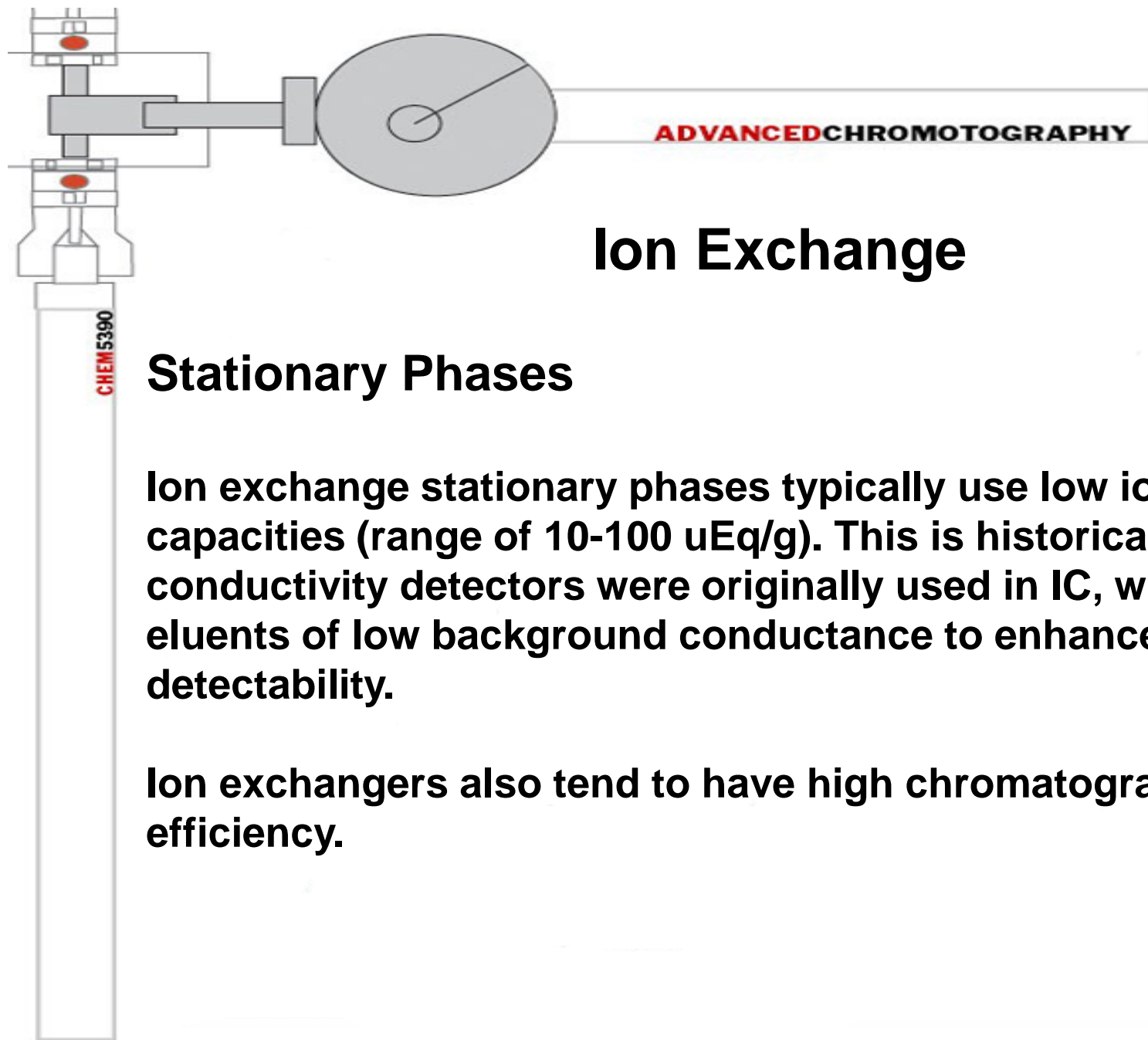
The suppressor requires a regenerant (or scavenger) solution to enable operation for extended periods.

IC w/o suppression

- high bkg conductivity**
- high linearity for calibration**
- low cost setup**
- can choose many eluents**

IC w/ suppression

- low bkg conductivity**
- non linear calibration**
- more expensive**
- eluent must react to H₂O**



Ion Exchange

Stationary Phases

Ion exchange stationary phases typically use low ion-exchange capacities (range of 10-100 $\mu\text{Eq/g}$). This is historically because conductivity detectors were originally used in IC, which prefer eluents of low background conductance to enhance detectability.

Ion exchangers also tend to have high chromatographic efficiency.

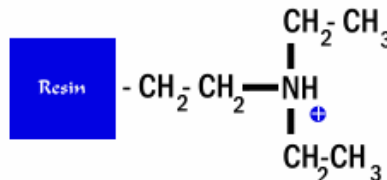
Ion Exchange

Stationary Phases

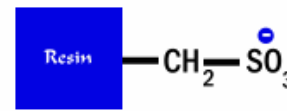
Ion exchangers have functional groups confined to a thin shell around the surface of the stationary phase particle to reduce the number of functional groups (ion-exchange capacity) and improve mass-transfer (chromatographic efficiency).



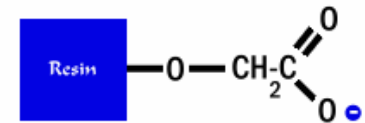
Q-anion exchanger



DEAE-anion exchanger



S-cation exchanger



CM-cation exchanger

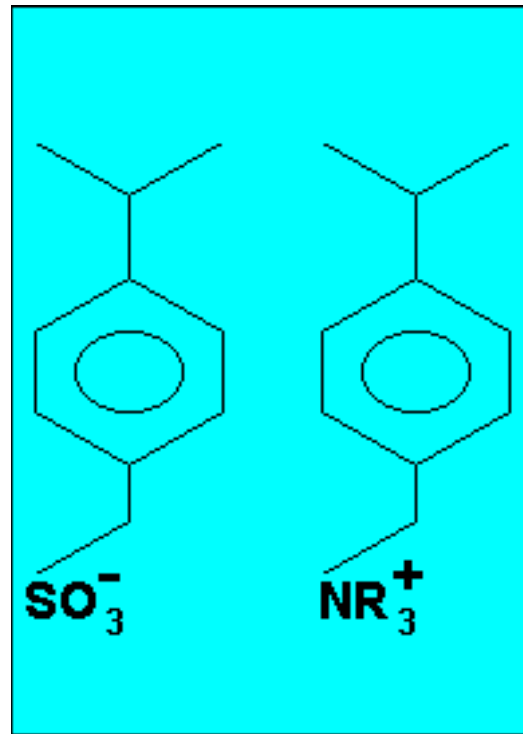
Ion Exchange

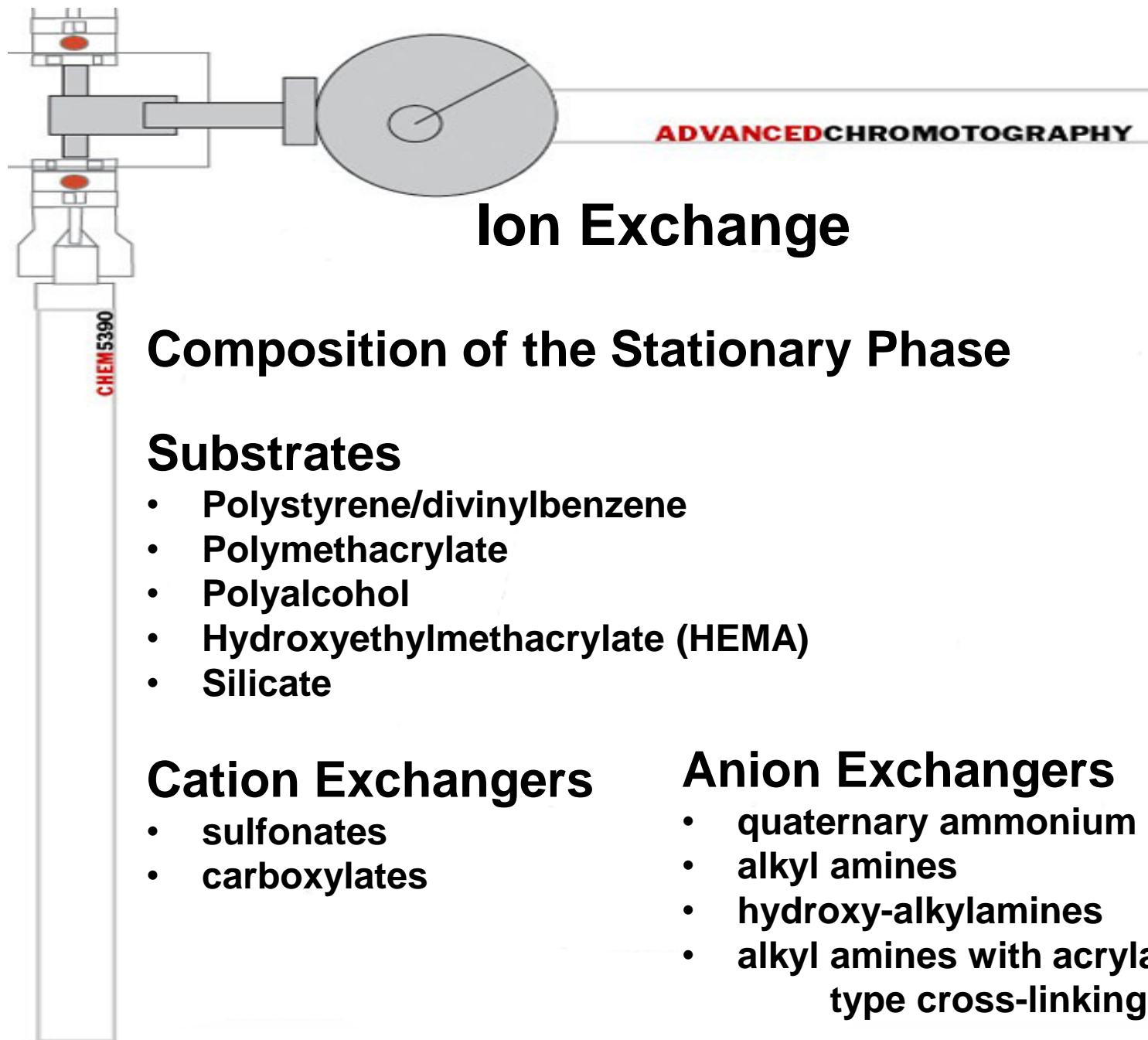
Composition of the Stationary Phase

substrate / resin carrier

spacer group
(alkyl chain)

group that carries the
separating capacity





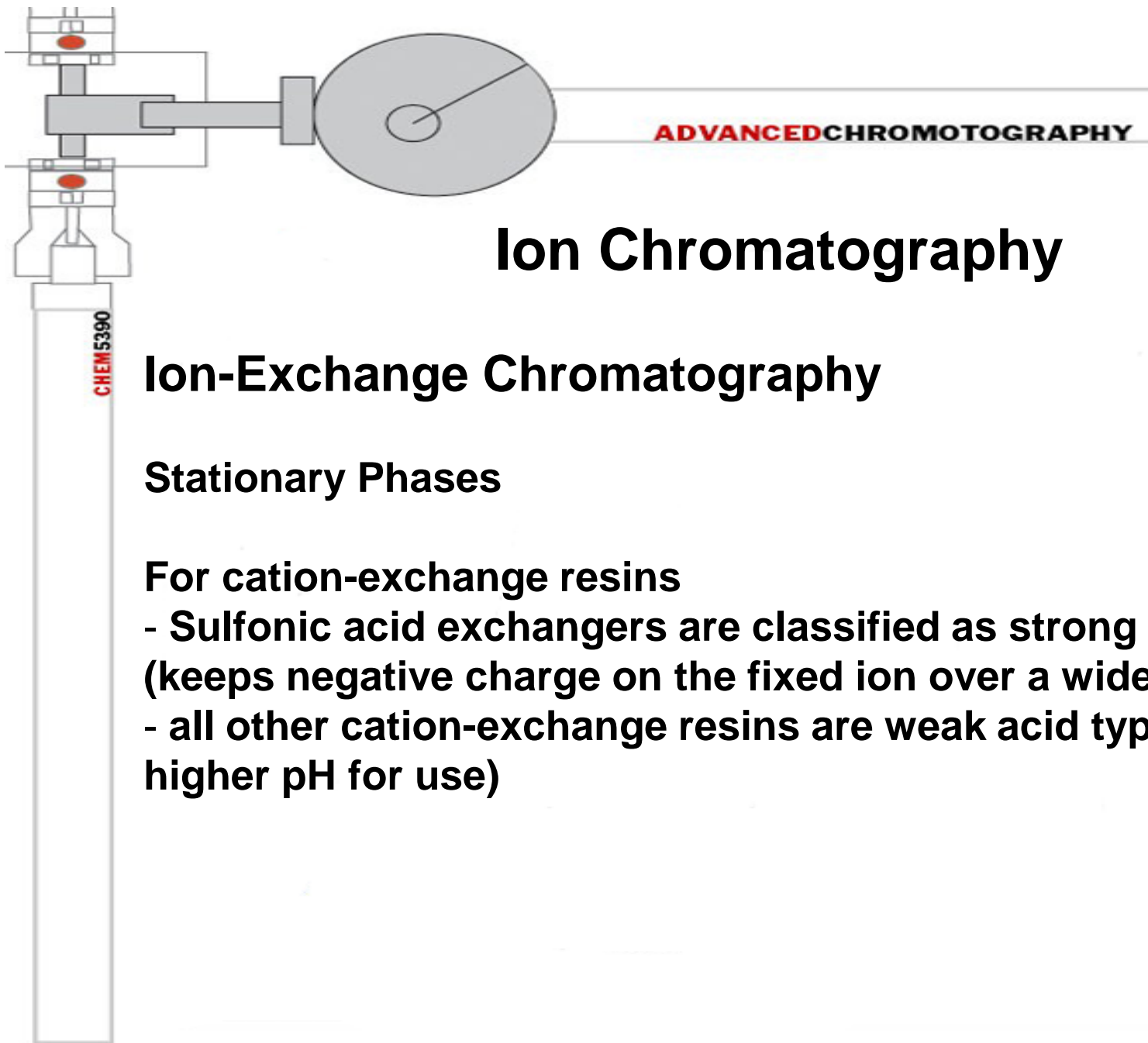
Ion Chromatography

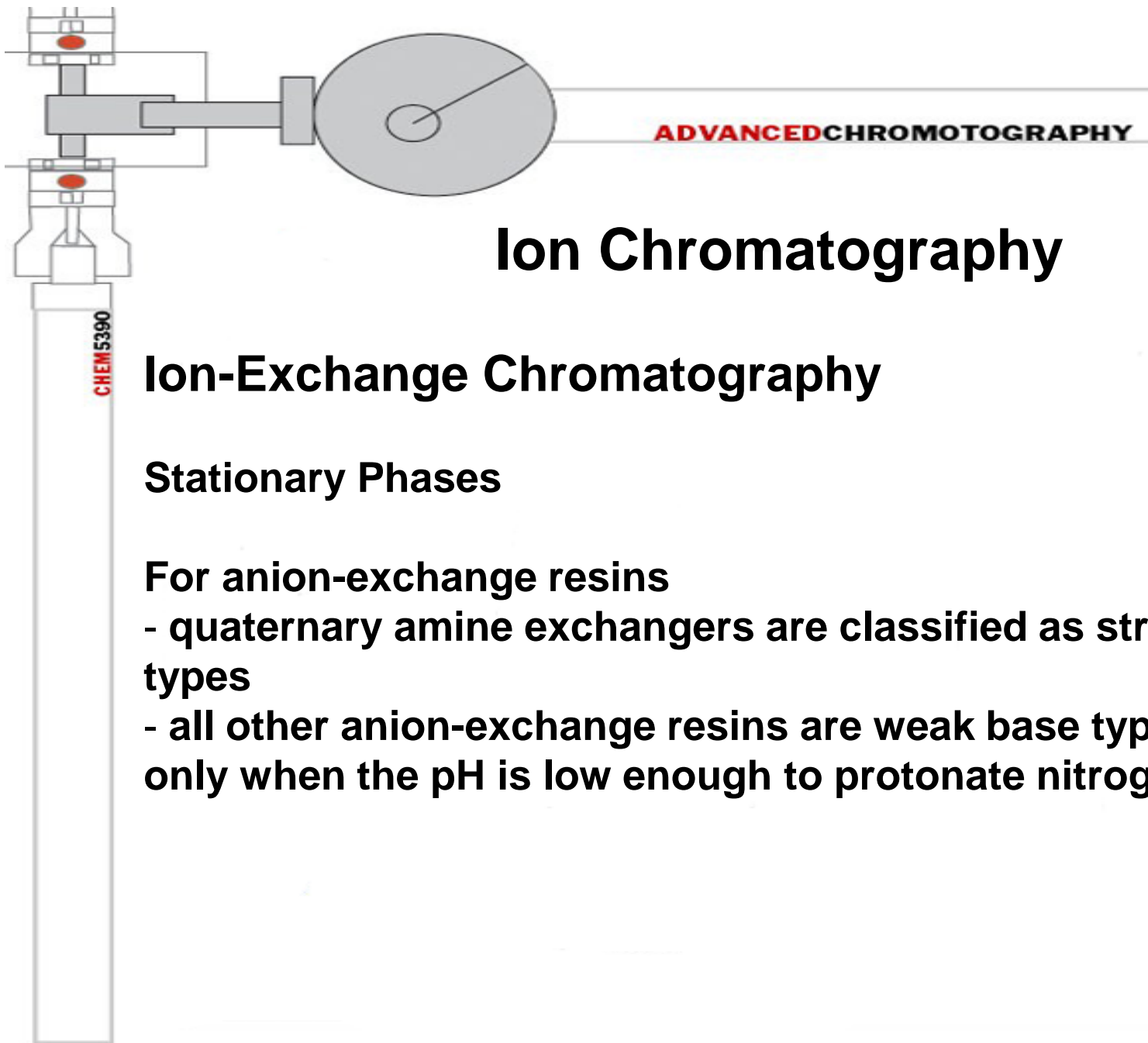
Ion-Exchange Chromatography

Stationary Phases

Table 6.12 Functional groups found on some typical synthetic ion-exchange materials.

Cation exchangers		Anion exchangers	
Type	Functional group	Type	Functional group
Sulfonic acid	$-\text{SO}_3^- \text{H}^+$	Quaternary amine	$-\text{N}(\text{CH}_3)_3^+ \text{OH}^-$
Carboxylic acid	$-\text{COO}^- \text{H}^+$	Quaternary amine	$-\text{N}(\text{CH}_3)_2(\text{EtOH})^+ \text{OH}^-$
Phosphonic acid	$-\text{PO}_3\text{H}^- \text{H}^+$	Tertiary amine	$-\text{NH}(\text{CH}_3)_2^+ \text{OH}^-$
Phosphinic acid	$-\text{PO}_2\text{H}^- \text{H}^+$	Secondary amine	$-\text{NH}_2(\text{CH}_3)^+ \text{OH}^-$
Phenolic	$-\text{O}^- \text{H}^+$	Primary amine	$-\text{NH}_3^+ \text{OH}^-$
Arsonic acid	$-\text{AsO}_3\text{H}^- \text{H}^+$		
Selenonic acid	$-\text{SeO}_3^- \text{H}^+$		





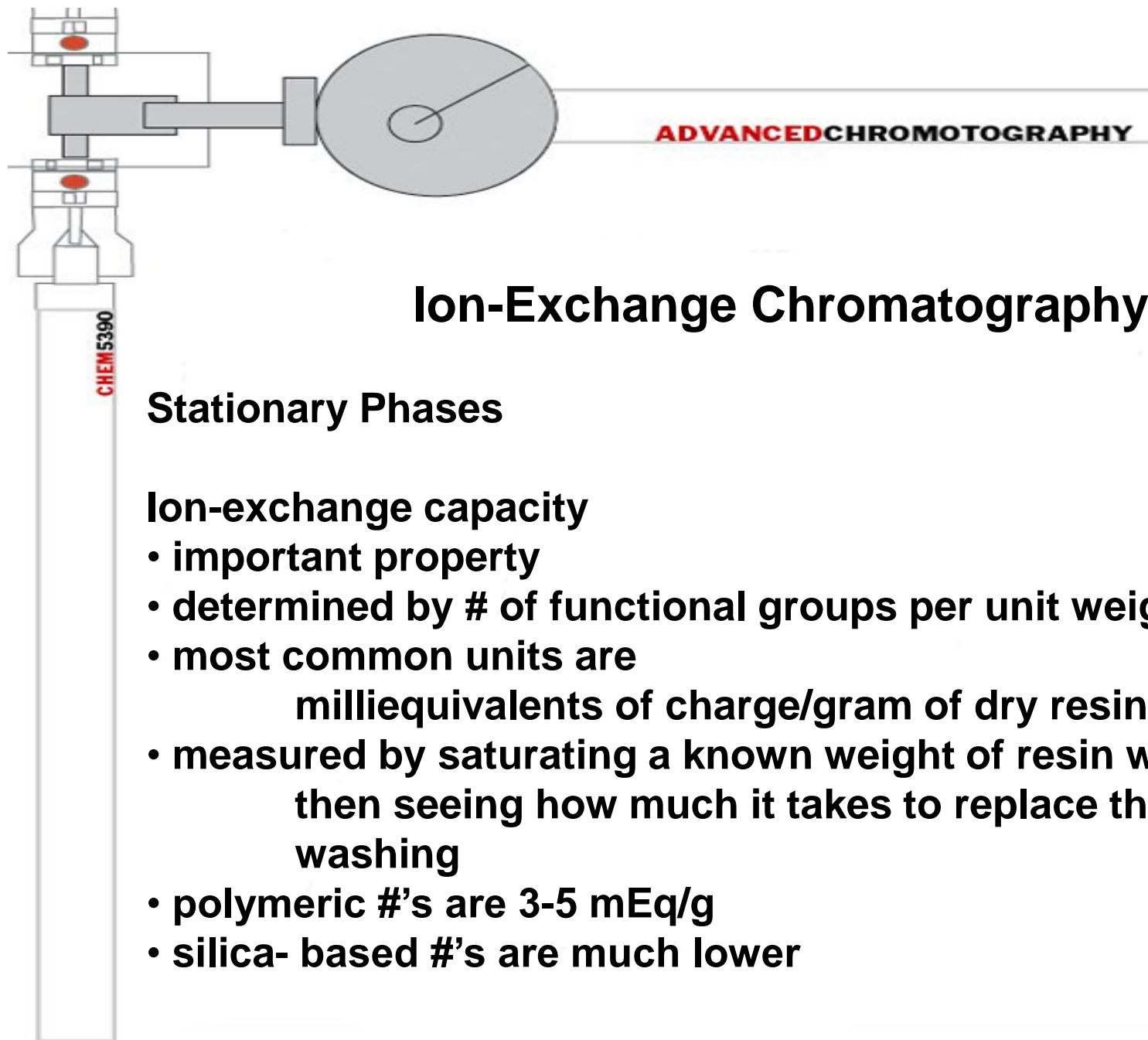
Ion Chromatography

Ion-Exchange Chromatography

Stationary Phases

For anion-exchange resins

- quaternary amine exchangers are classified as strong base types**
- all other anion-exchange resins are weak base types (functions only when the pH is low enough to protonate nitrogen)**

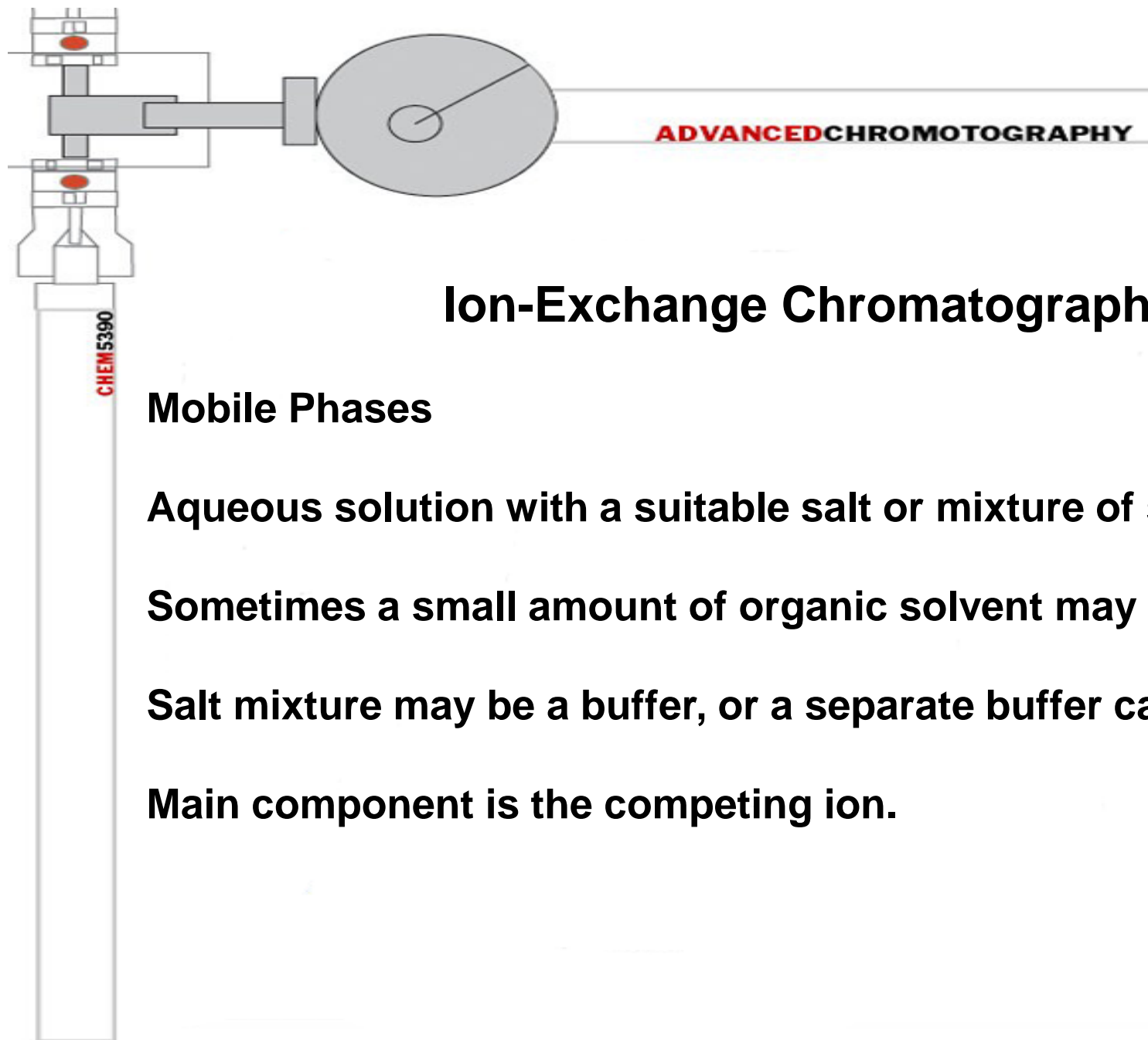


Ion-Exchange Chromatography

Stationary Phases

Ion-exchange capacity

- **important property**
- **determined by # of functional groups per unit weight of resin**
- **most common units are**
 milliequivalents of charge/gram of dry resin
- **measured by saturating a known weight of resin with an ion, then seeing how much it takes to replace the ion during washing**
- **polymeric #'s are 3-5 mEq/g**
- **silica- based #'s are much lower**



Ion-Exchange Chromatography

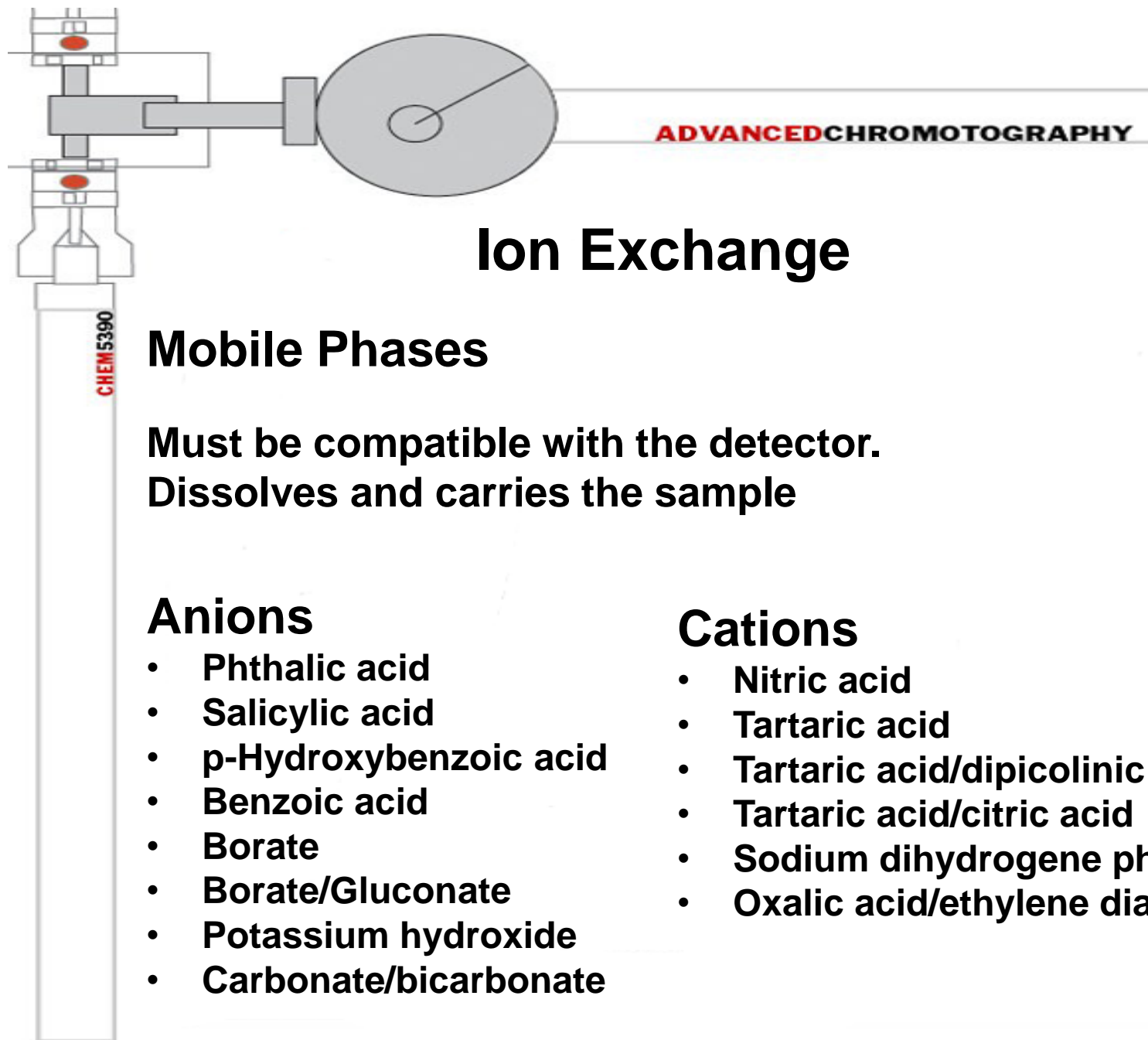
Mobile Phases

Aqueous solution with a suitable salt or mixture of salts.

Sometimes a small amount of organic solvent may be present.

Salt mixture may be a buffer, or a separate buffer can be added.

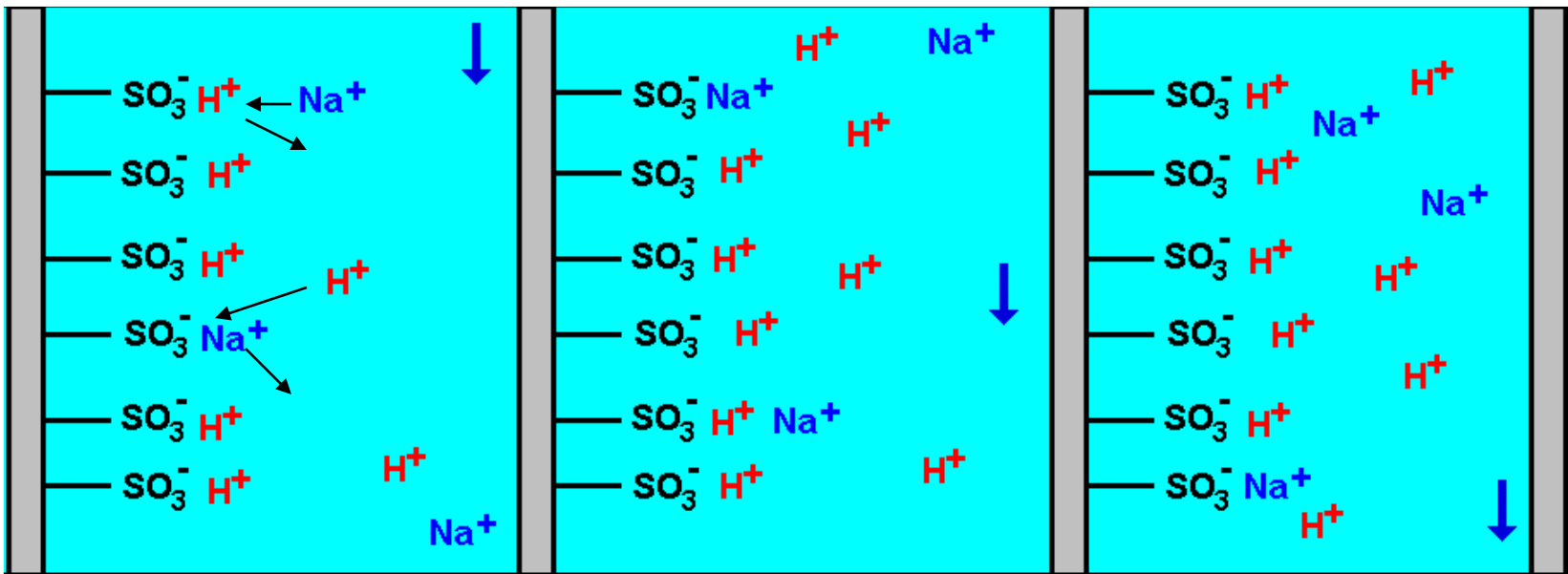
Main component is the competing ion.



Ion Exchange

Cation Separation Mechanism

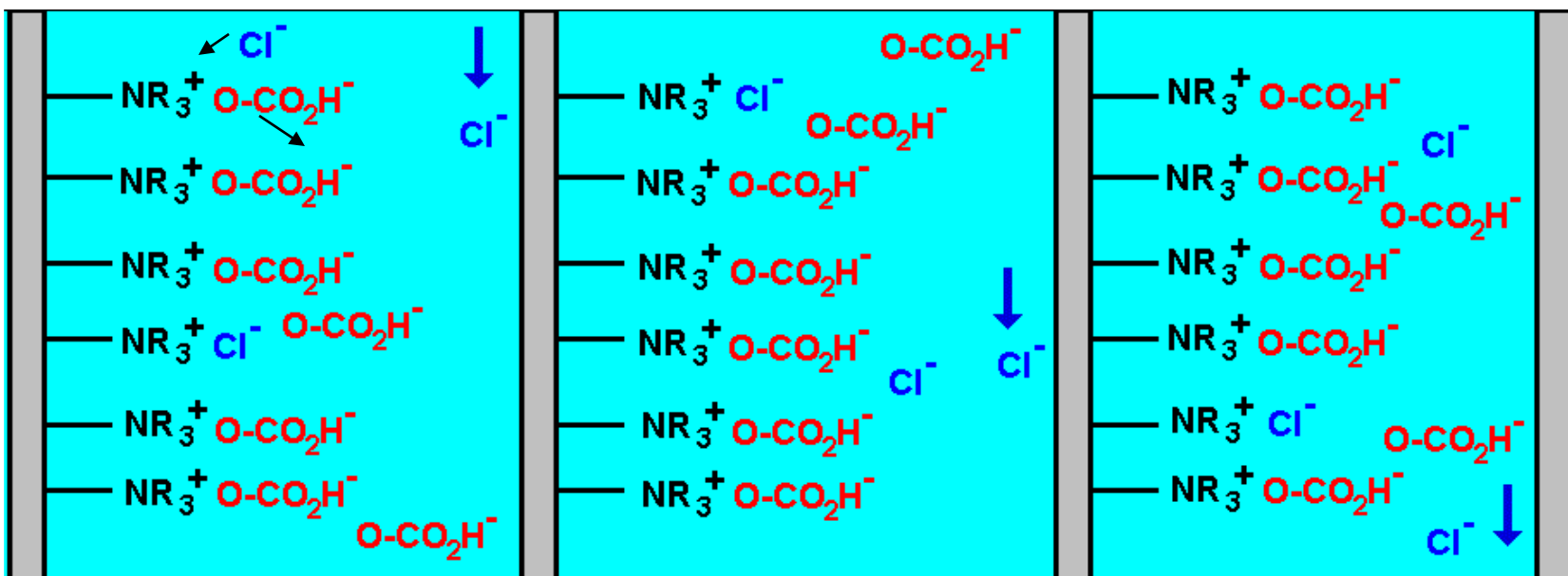
Stationary phase and mobile phase compete for the analyte

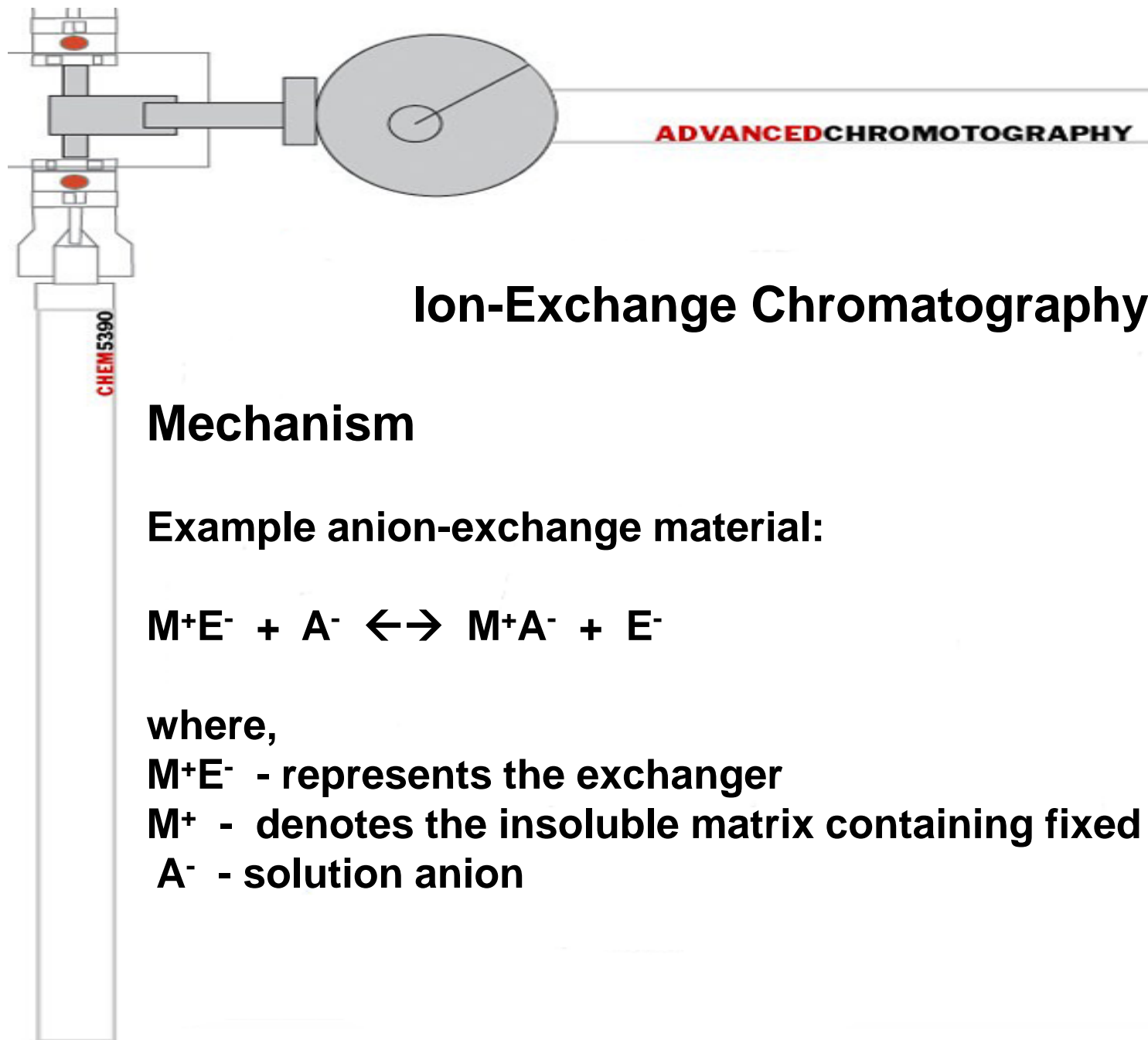


Ion Exchange

Anion Separation Mechanism

Stationary phase and mobile phase compete for the analyte





Ion-Exchange Chromatography

Mechanism

Example anion-exchange material:

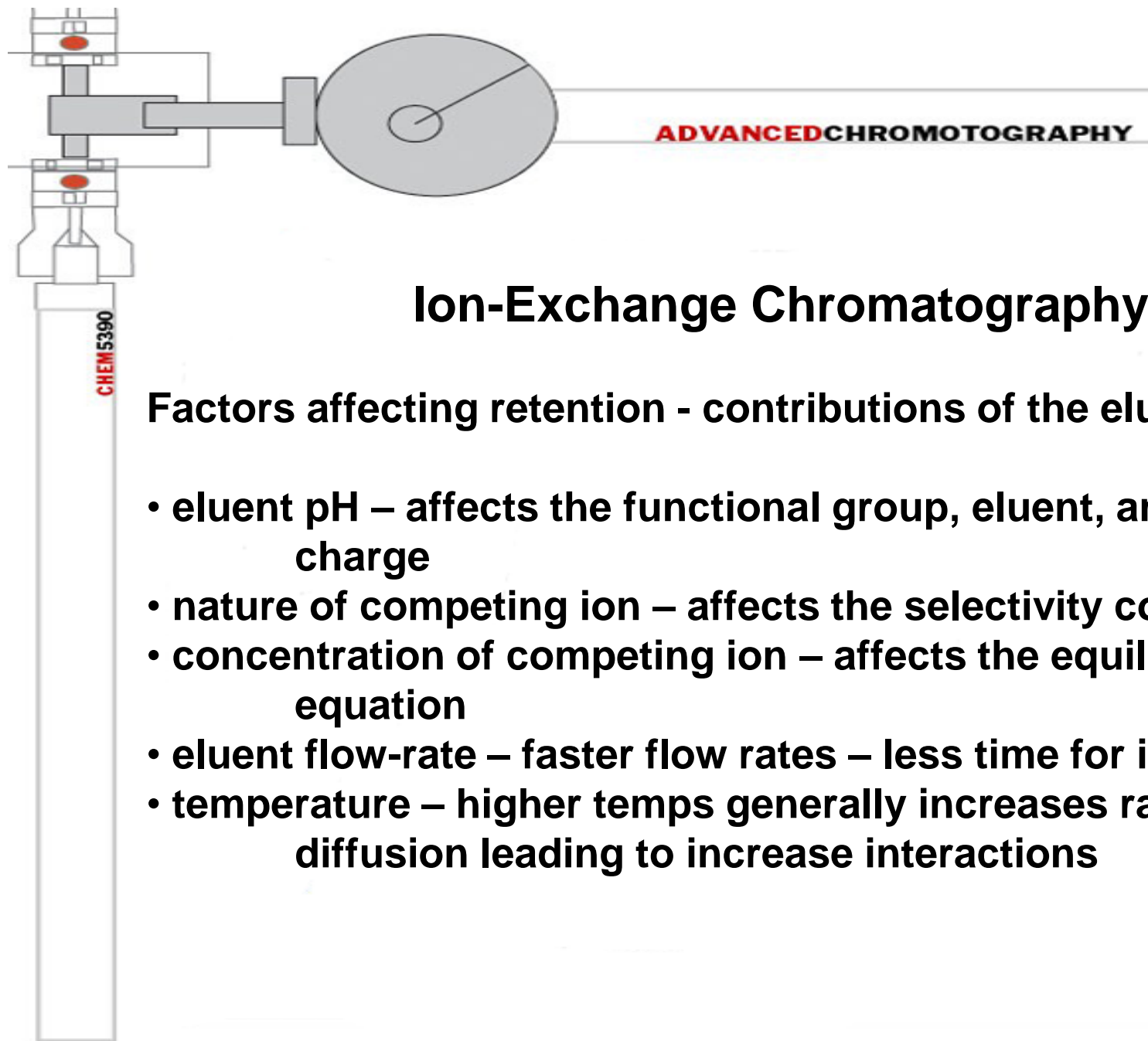


where,

M⁺E⁻ - represents the exchanger

M⁺ - denotes the insoluble matrix containing fixed + ion

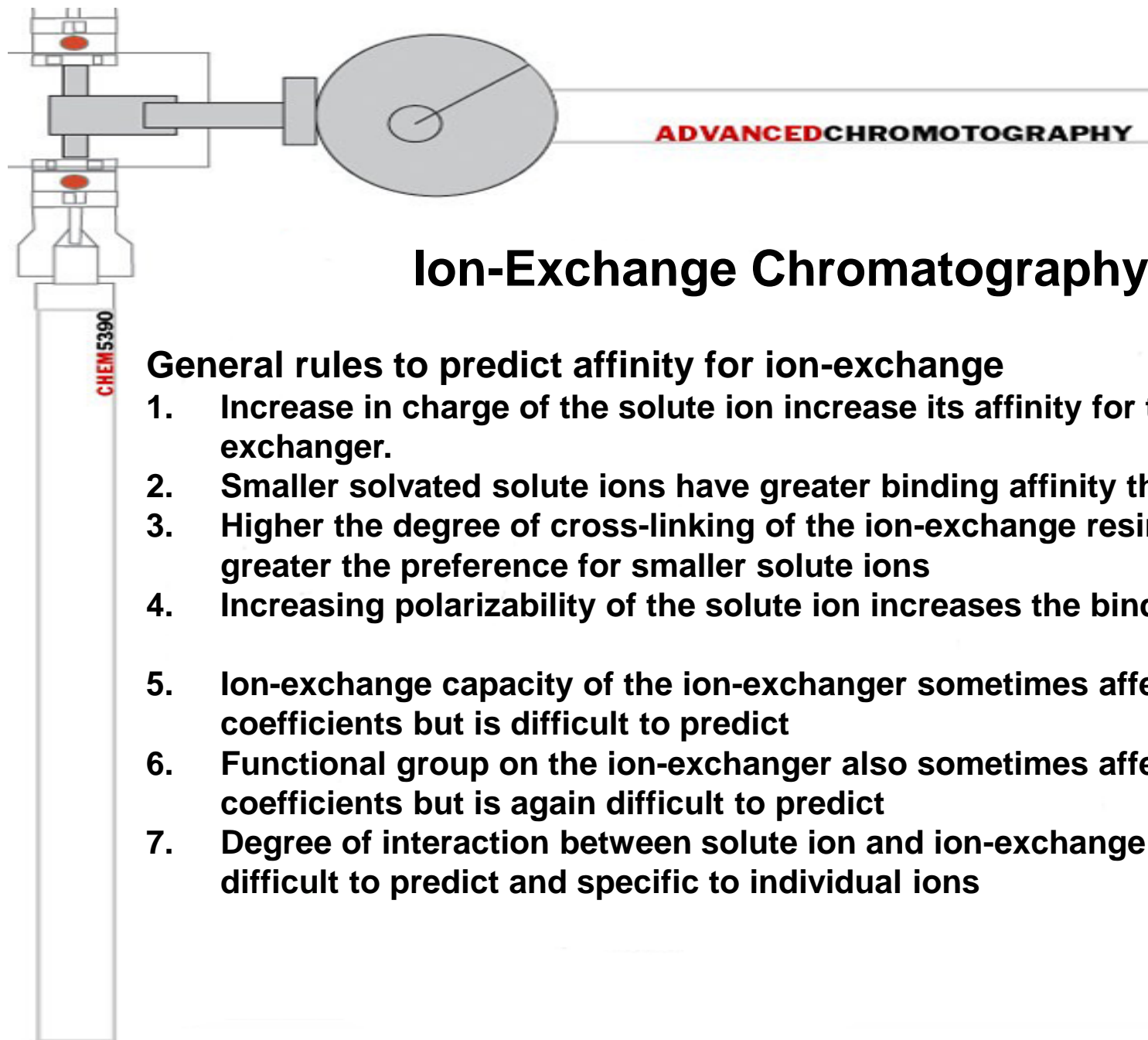
A⁻ - solution anion

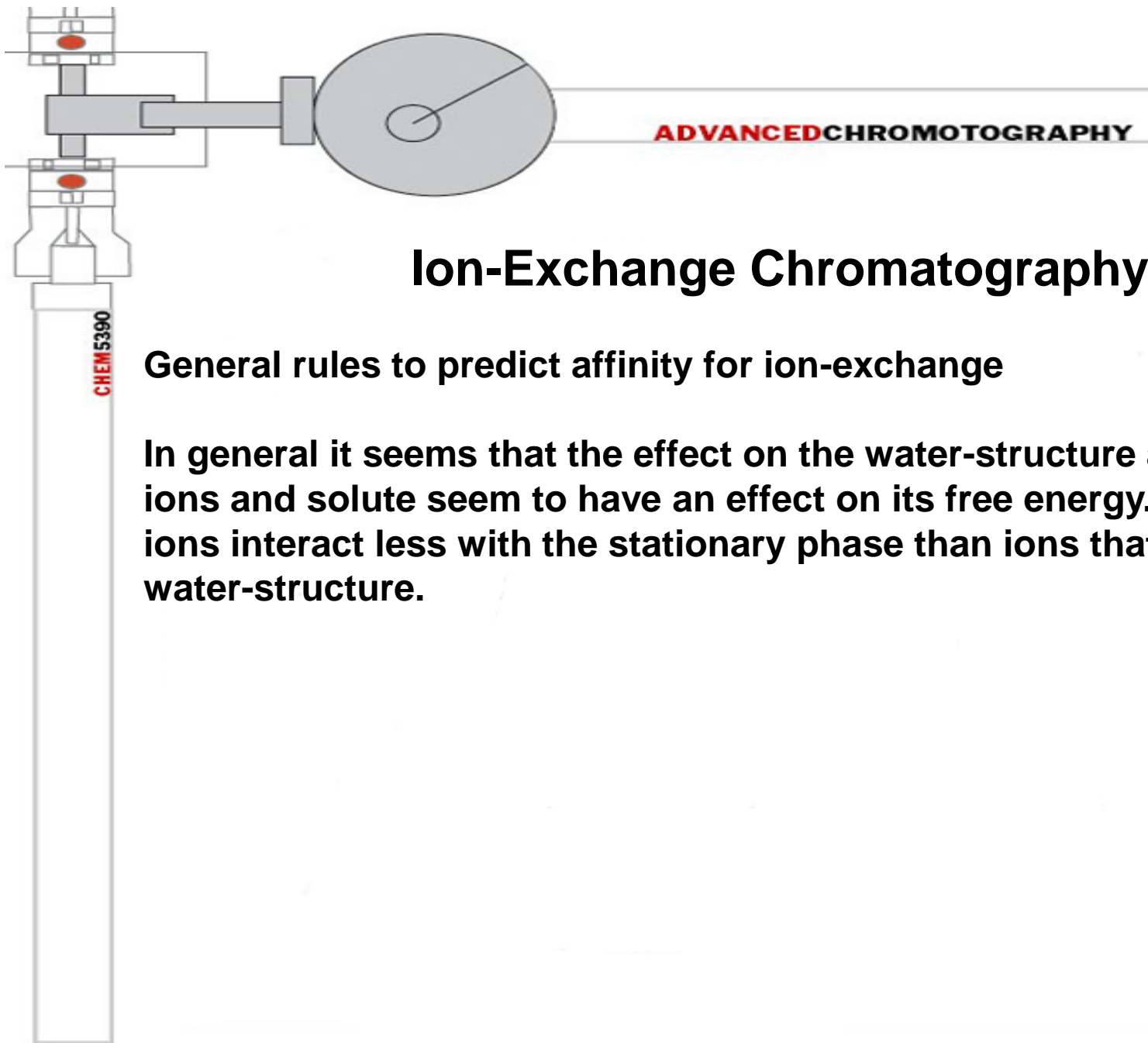


Ion-Exchange Chromatography

Factors affecting retention - contributions of the eluent

- **eluent pH – affects the functional group, eluent, and solute ion charge**
- **nature of competing ion – affects the selectivity coefficient**
- **concentration of competing ion – affects the equilibrium equation**
- **eluent flow-rate – faster flow rates – less time for interactions**
- **temperature – higher temps generally increases rate of diffusion leading to increase interactions**

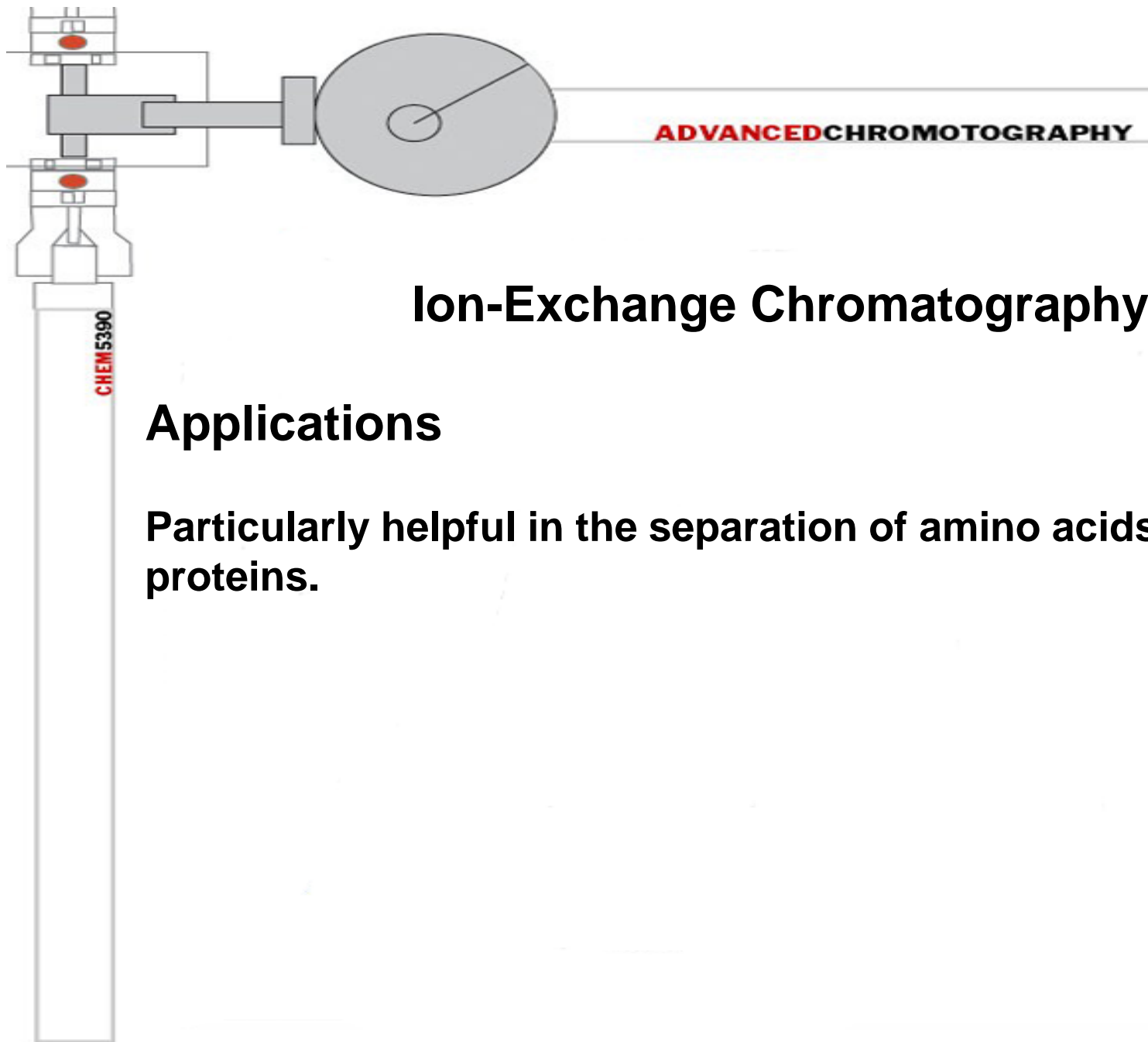




Ion-Exchange Chromatography

General rules to predict affinity for ion-exchange

In general it seems that the effect on the water-structure around the ions and solute seem to have an effect on its free energy. Well solvated ions interact less with the stationary phase than ions that disrupt the water-structure.



Ion-Exchange Chromatography

Applications

Particularly helpful in the separation of amino acids and proteins.

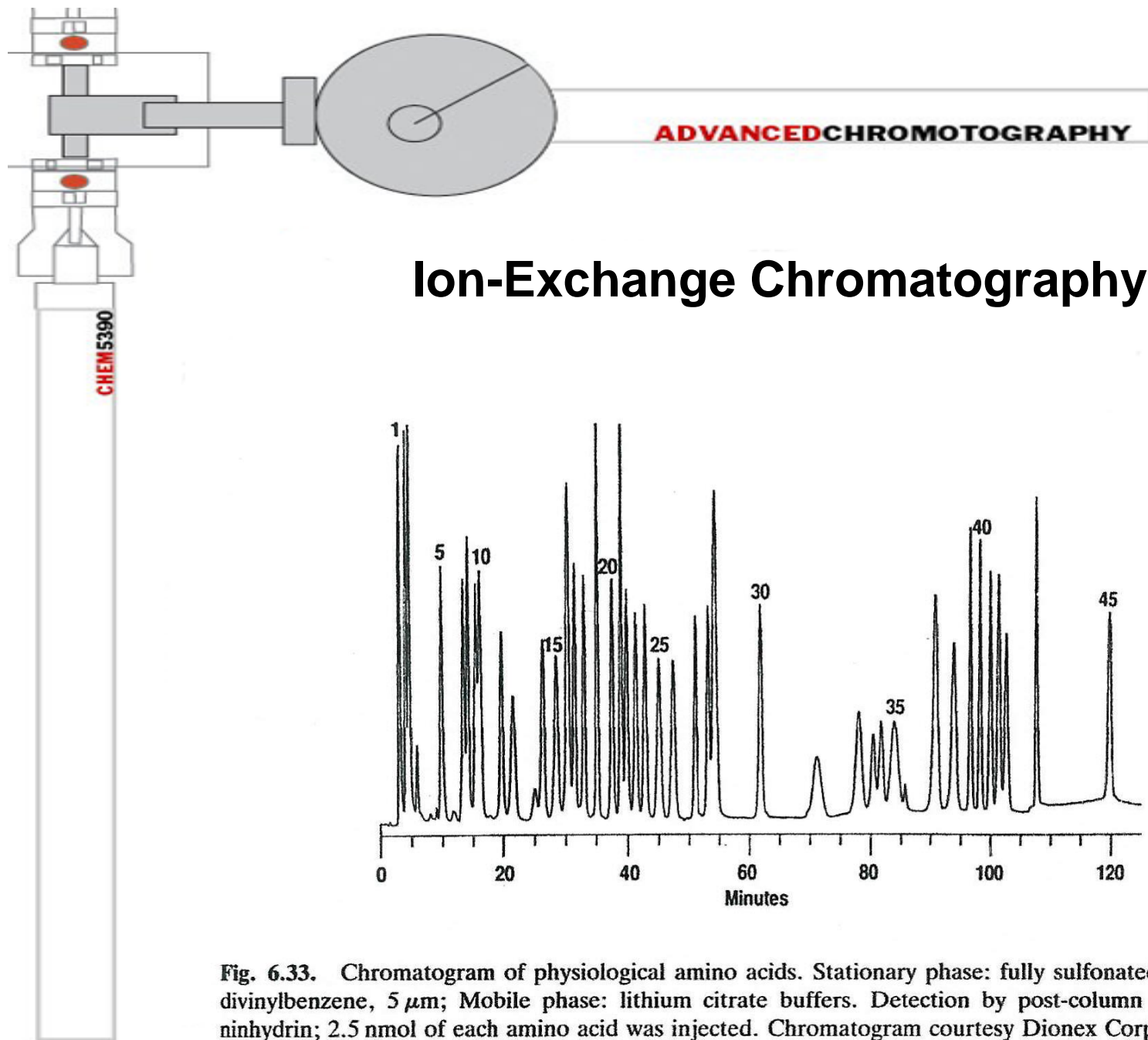


Fig. 6.33. Chromatogram of physiological amino acids. Stationary phase: fully sulfonated polystyrene-divinylbenzene, 5 μm ; Mobile phase: lithium citrate buffers. Detection by post-column reaction with ninhydrin; 2.5 nmol of each amino acid was injected. Chromatogram courtesy Dionex Corporation.

Ion Exchange

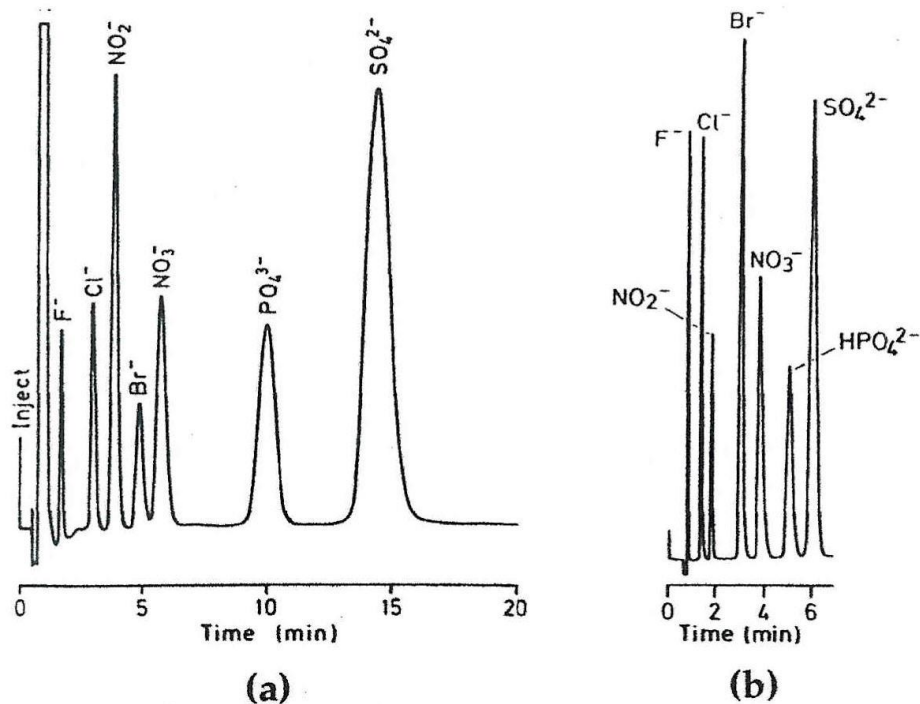
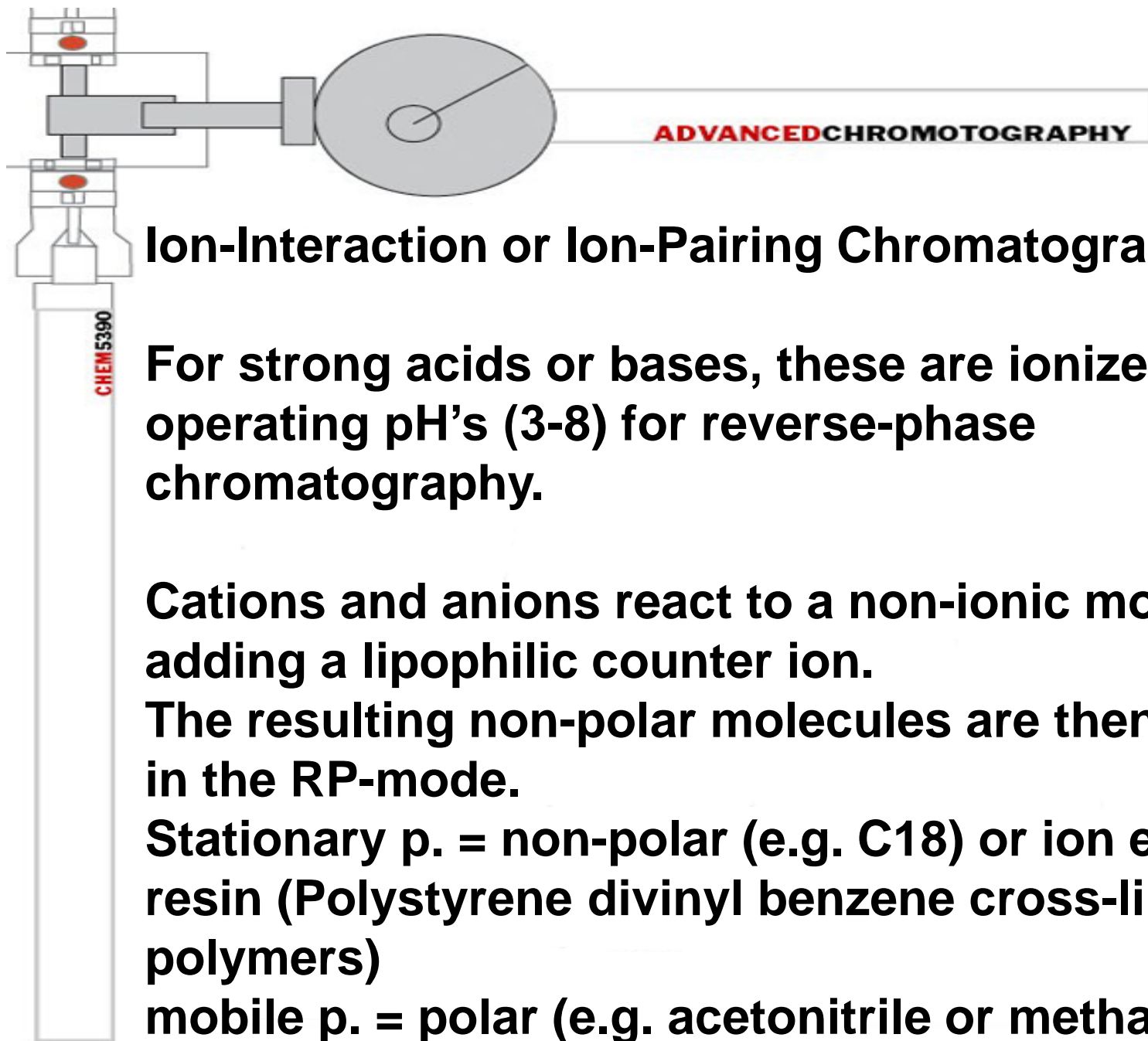


Fig. 6.39. Anion separations obtained using (a) nonsuppressed and (b) suppressed ion chromatography. (a) A Waters IC Pak A column was used with gluconate-borate eluent. (b) A Dionex HPIC-AS4A column was used with a carbonate-bicarbonate eluent. Conductivity detection was employed in both cases. Chromatograms courtesy of Waters and Dionex.



Ion-Interaction or Ion-Pairing Chromatography

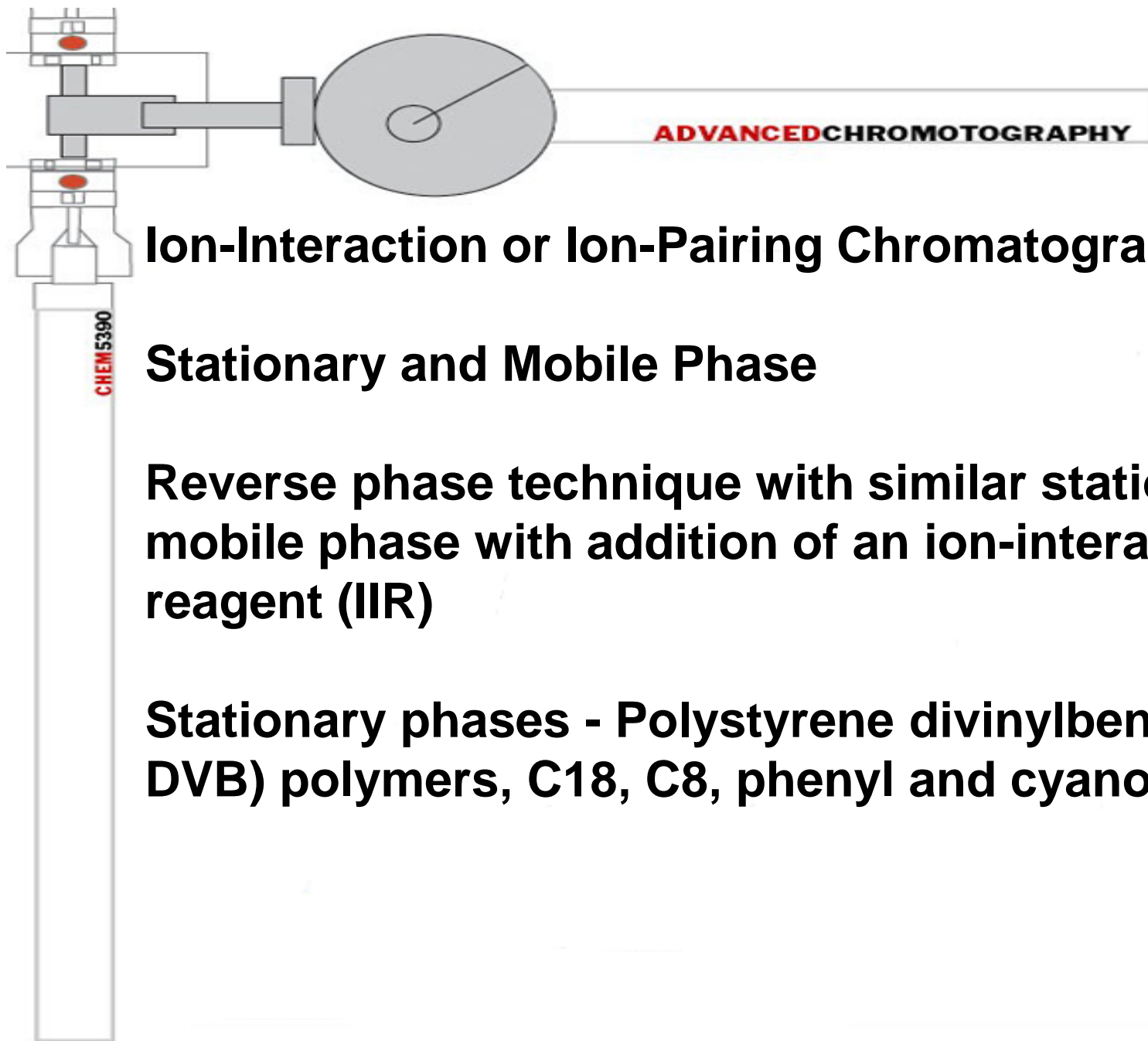
For strong acids or bases, these are ionized at operating pH's (3-8) for reverse-phase chromatography.

Cations and anions react to a non-ionic molecule by adding a lipophilic counter ion.

The resulting non-polar molecules are then separated in the RP-mode.

Stationary p. = non-polar (e.g. C18) or ion exchange resin (Polystyrene divinyl benzene cross-linked polymers)

mobile p. = polar (e.g. acetonitrile or methanol/water)

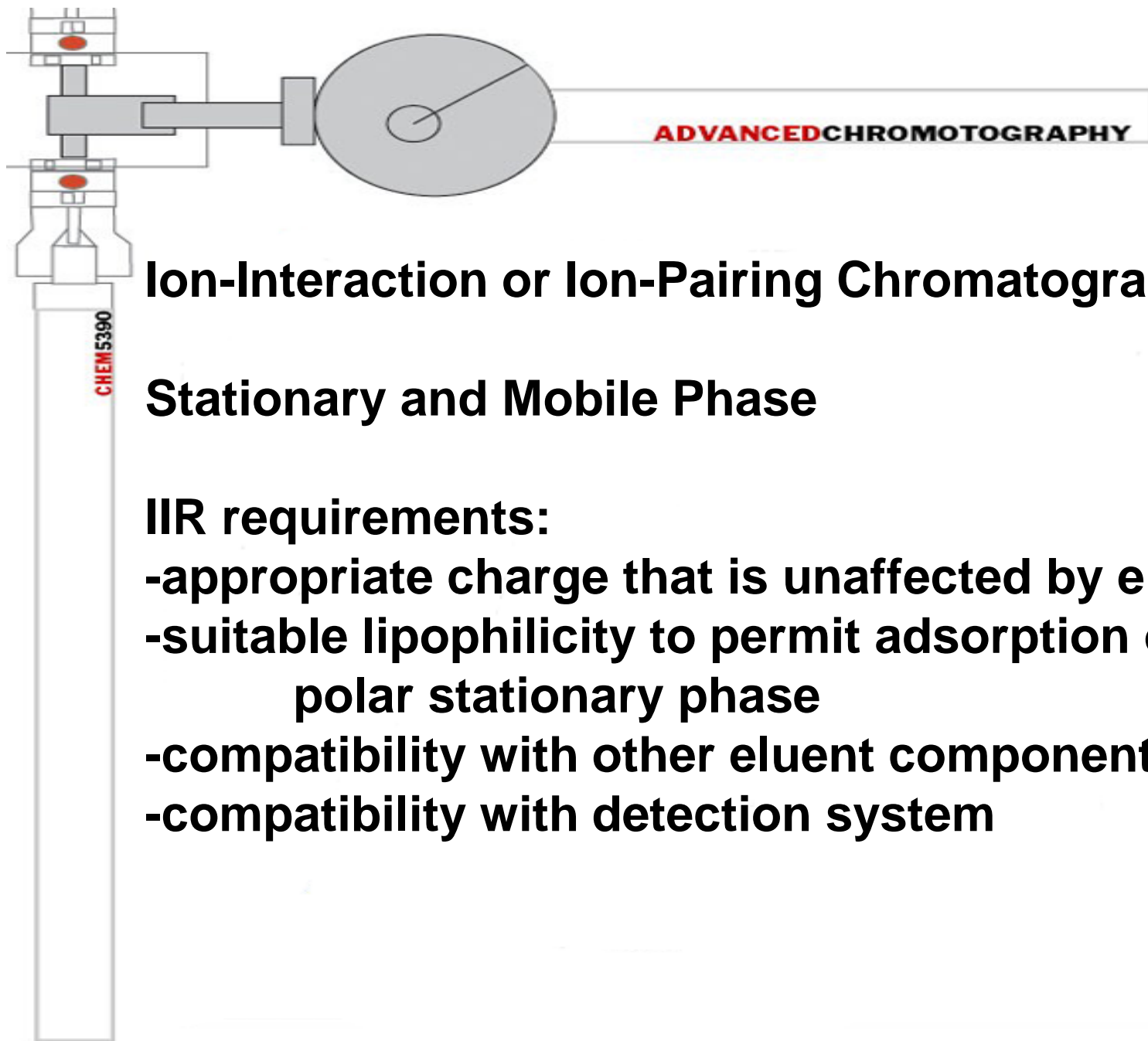


Ion-Interaction or Ion-Pairing Chromatography

Stationary and Mobile Phase

Reverse phase technique with similar stationary and mobile phase with addition of an ion-interacting reagent (IIR)

Stationary phases - Polystyrene divinylbenzene (PS-DVB) polymers, C18, C8, phenyl and cyano groups.

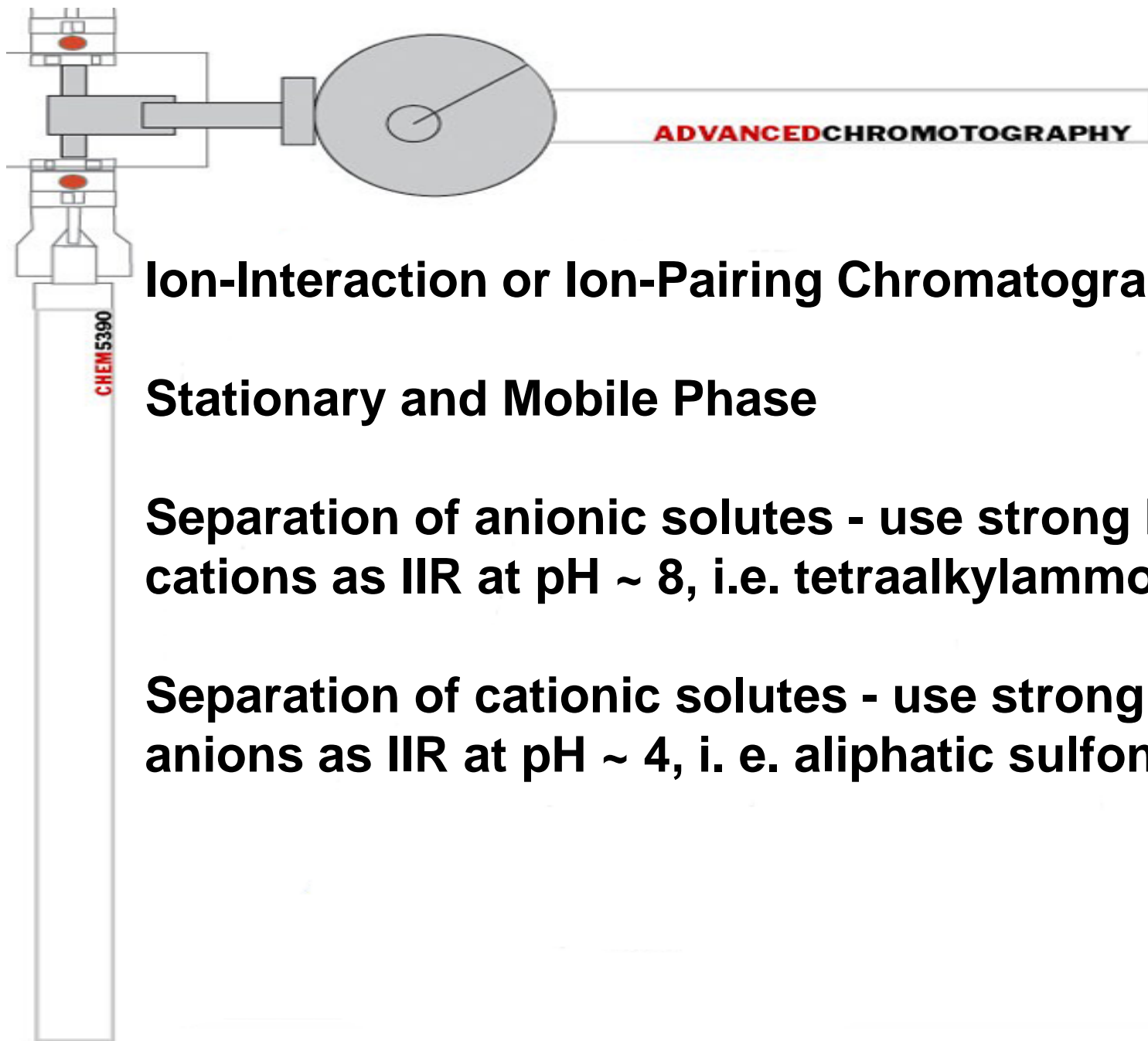


Ion-Interaction or Ion-Pairing Chromatography

Stationary and Mobile Phase

IIR requirements:

- appropriate charge that is unaffected by eluent pH**
- suitable lipophilicity to permit adsorption onto non-polar stationary phase**
- compatibility with other eluent components**
- compatibility with detection system**

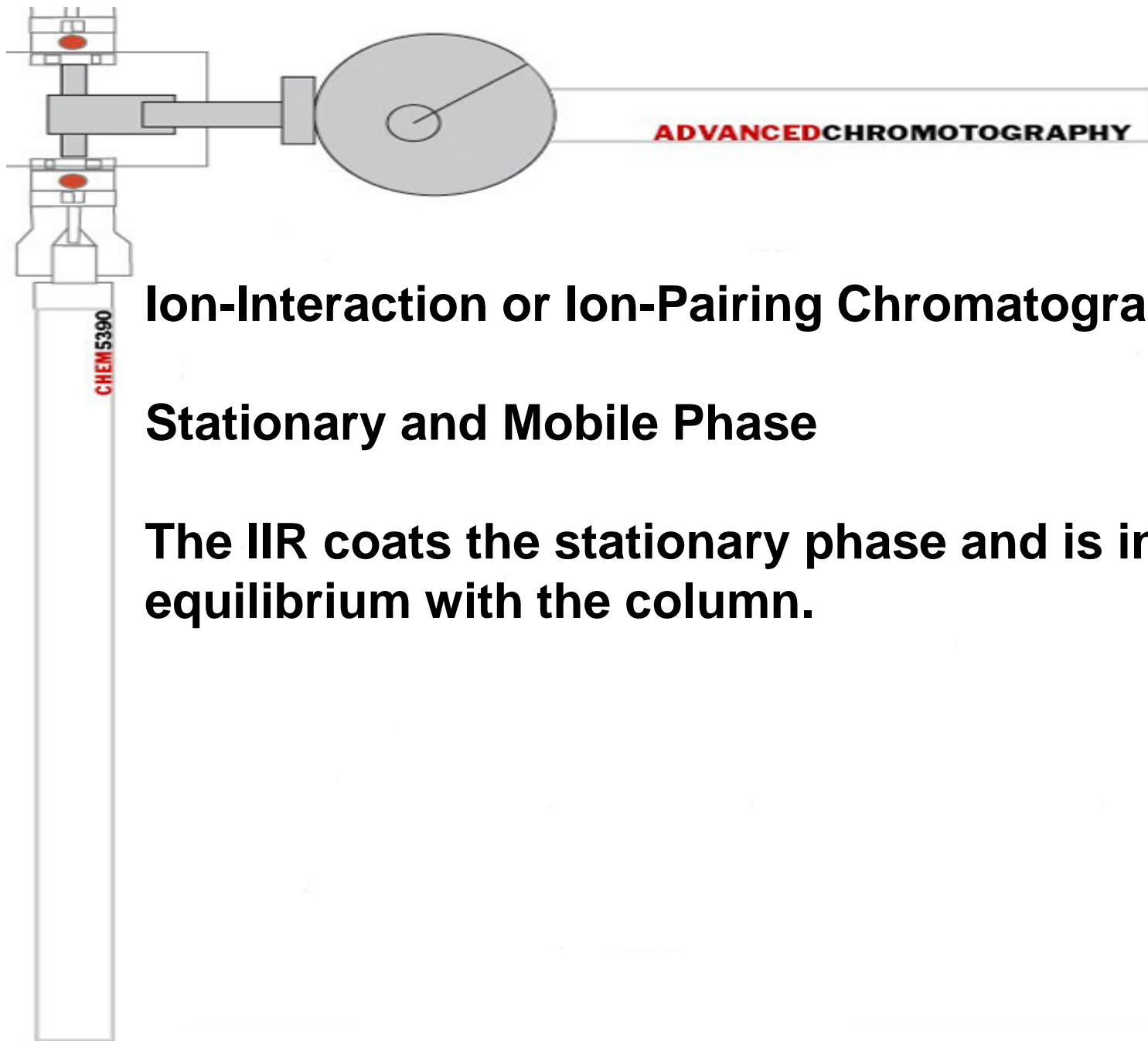


Ion-Interaction or Ion-Pairing Chromatography

Stationary and Mobile Phase

Separation of anionic solutes - use strong base cations as IIR at pH ~ 8, i.e. tetraalkylammonium ions

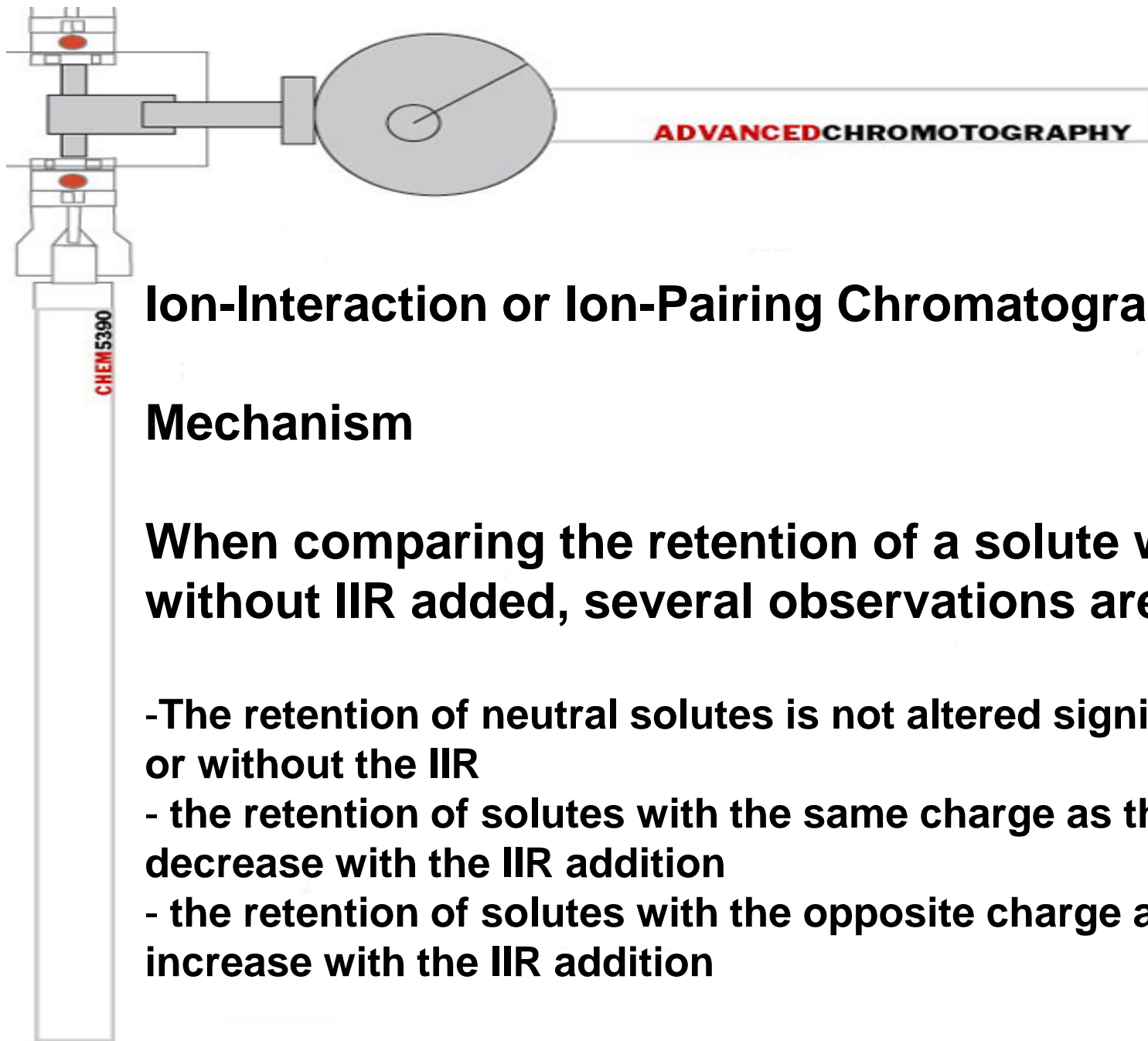
Separation of cationic solutes - use strong acid anions as IIR at pH ~ 4, i. e. aliphatic sulfonate ions.



Ion-Interaction or Ion-Pairing Chromatography

Stationary and Mobile Phase

The IIR coats the stationary phase and is in equilibrium with the column.

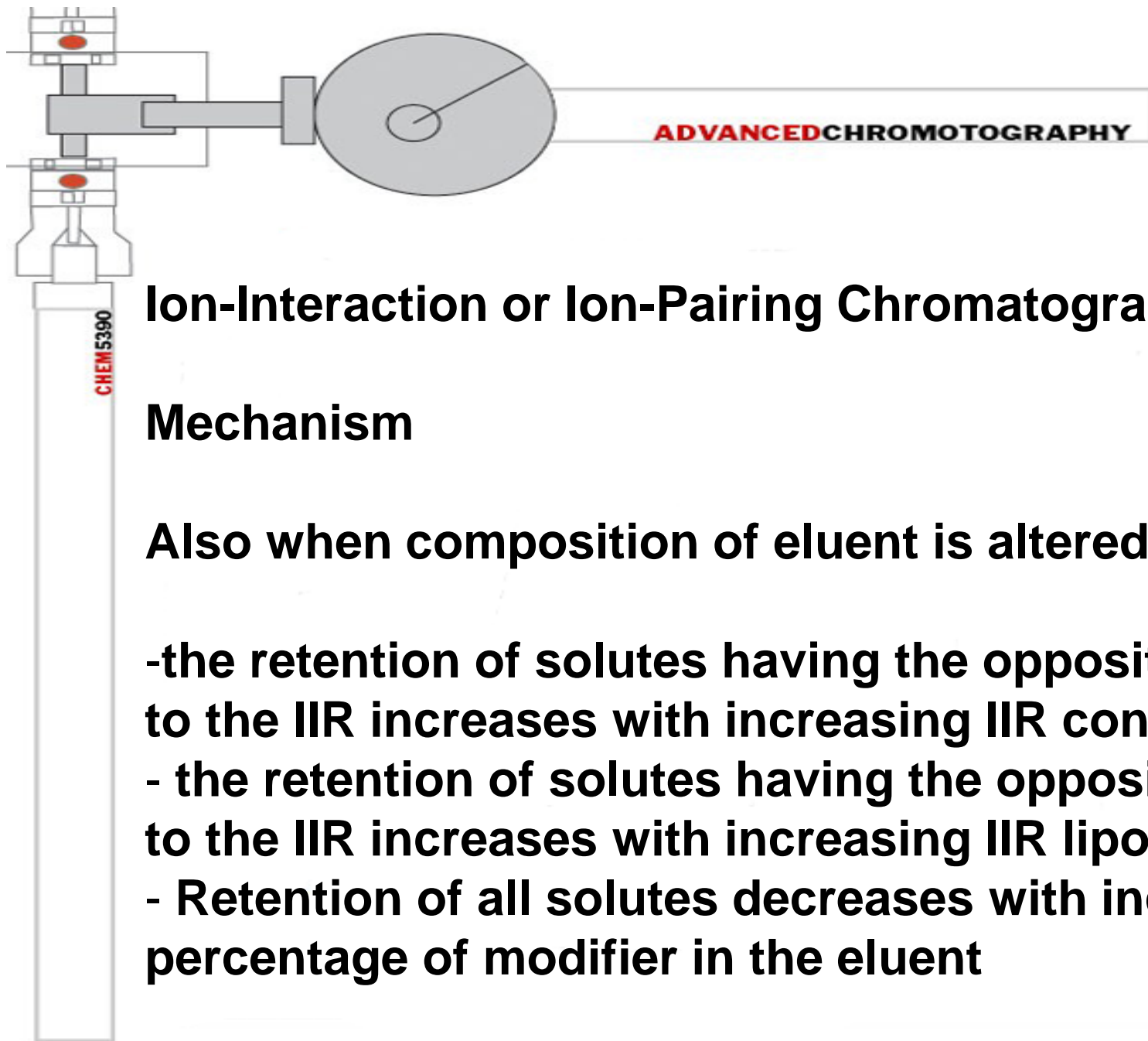


Ion-Interaction or Ion-Pairing Chromatography

Mechanism

When comparing the retention of a solute with and without IIR added, several observations are made;

- The retention of neutral solutes is not altered significantly with or without the IIR**
- the retention of solutes with the same charge as the IIR decrease with the IIR addition**
- the retention of solutes with the opposite charge as the IIR increase with the IIR addition**

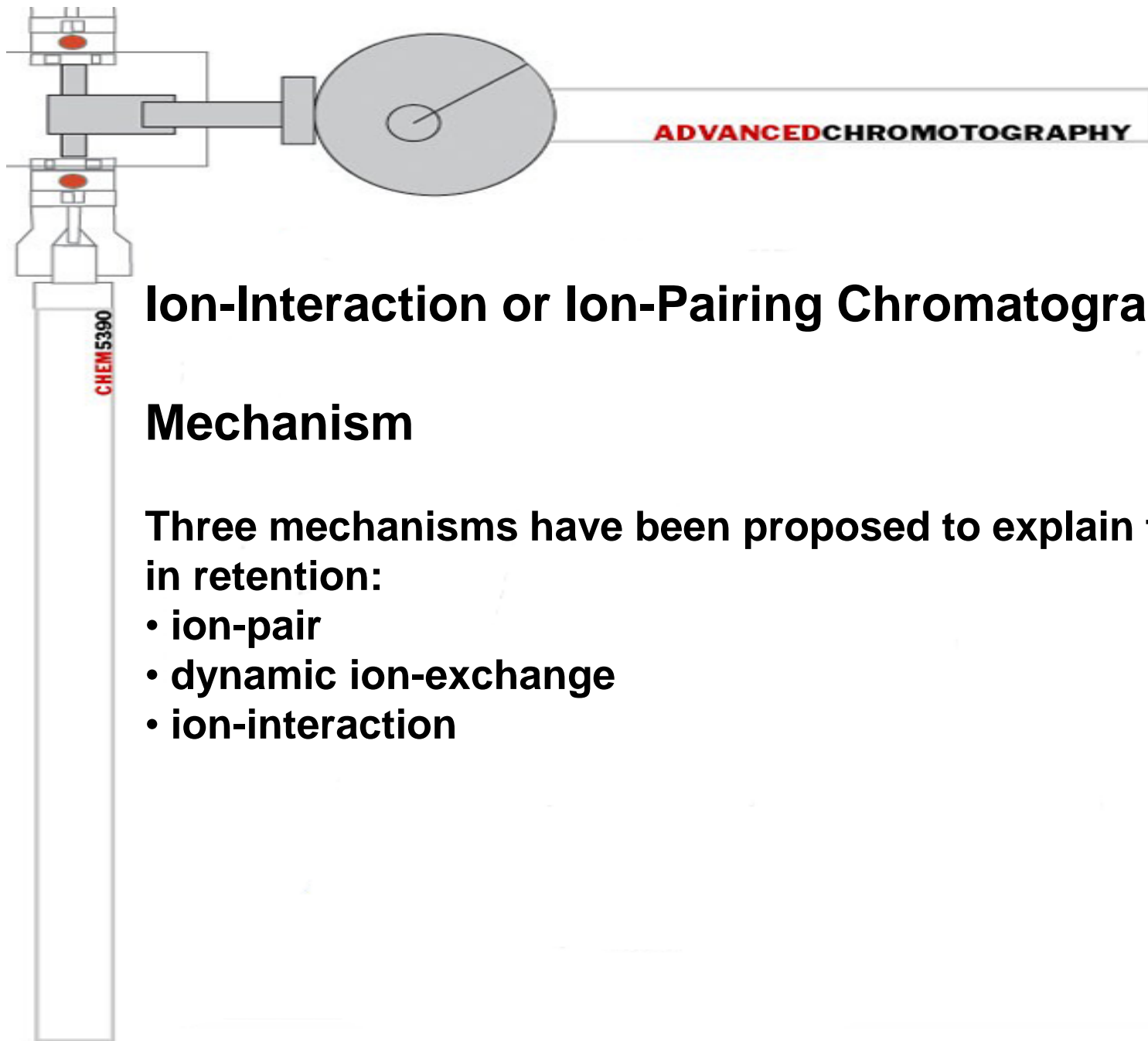


Ion-Interaction or Ion-Pairing Chromatography

Mechanism

Also when composition of eluent is altered;

- the retention of solutes having the opposite charge to the IIR increases with increasing IIR concentration**
- the retention of solutes having the opposite charge to the IIR increases with increasing IIR lipophilicity**
- Retention of all solutes decreases with increasing percentage of modifier in the eluent**

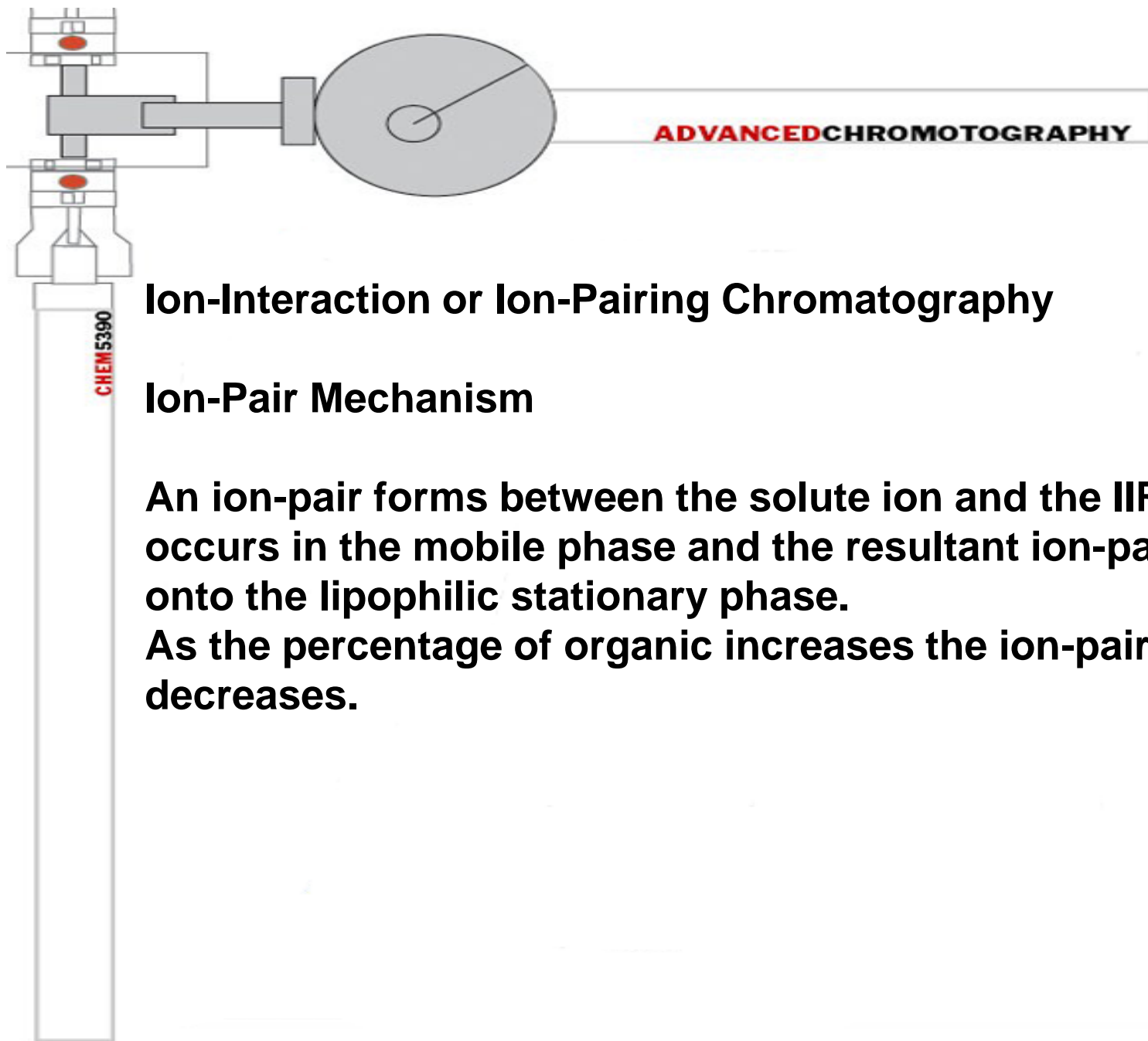


Ion-Interaction or Ion-Pairing Chromatography

Mechanism

Three mechanisms have been proposed to explain the changes in retention:

- ion-pair**
- dynamic ion-exchange**
- ion-interaction**



Ion-Interaction or Ion-Pairing Chromatography

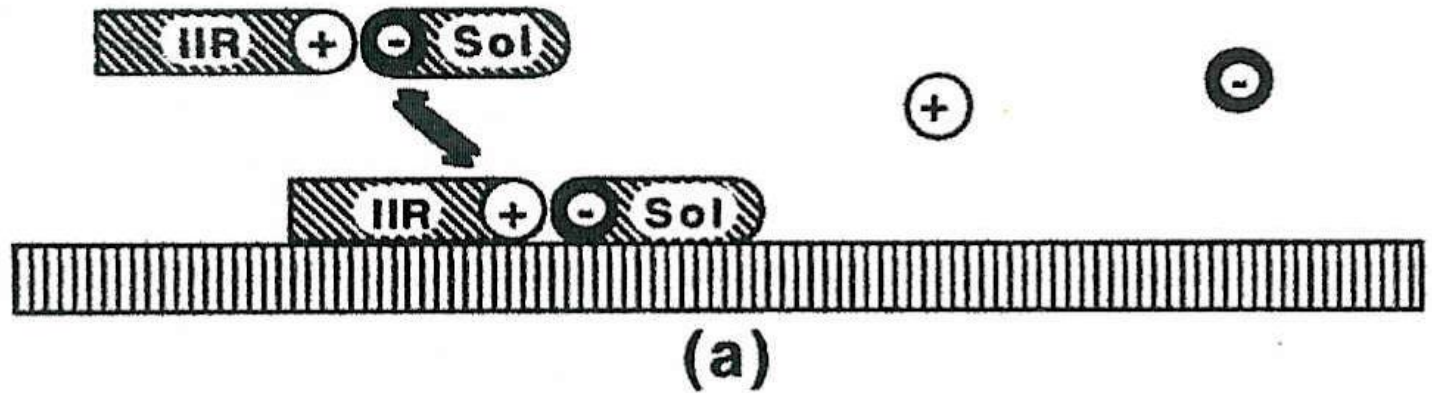
Ion-Pair Mechanism

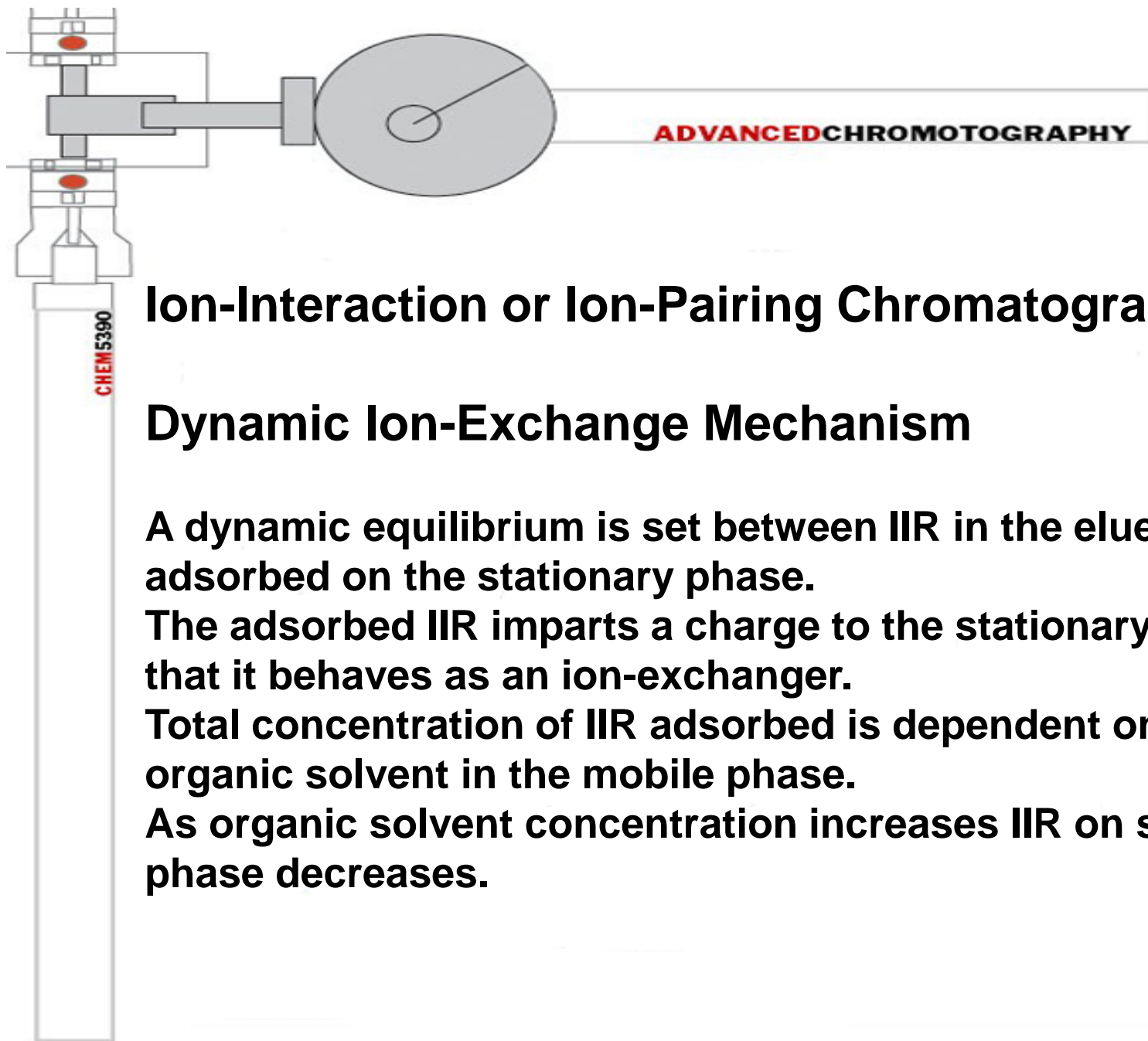
An ion-pair forms between the solute ion and the IIR. This occurs in the mobile phase and the resultant ion-pair partitions onto the lipophilic stationary phase.

As the percentage of organic increases the ion-pair retention decreases.

ADVANCEDCHROMATOGRAPHY

Ion Chromatography





Ion-Interaction or Ion-Pairing Chromatography

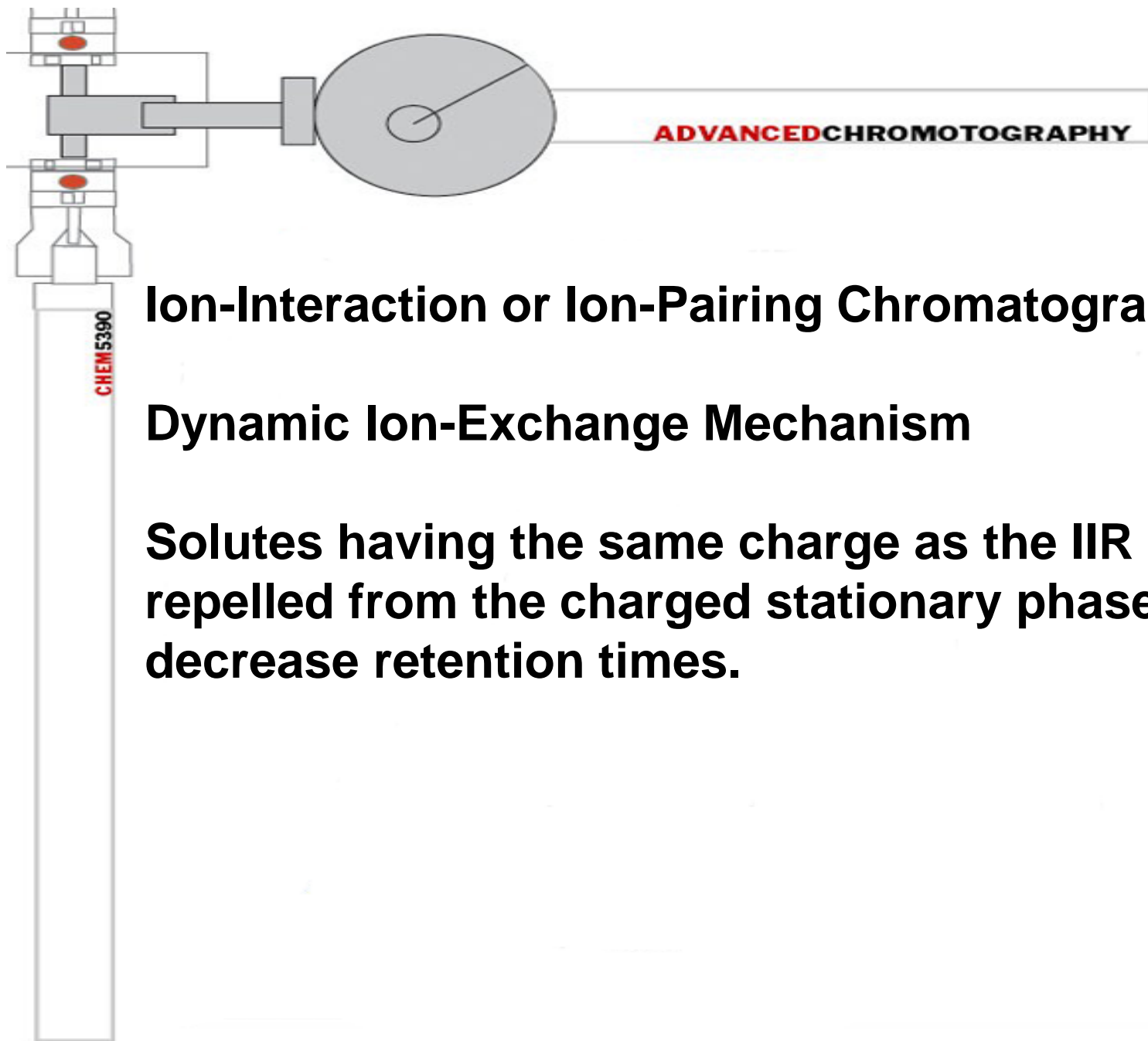
Dynamic Ion-Exchange Mechanism

A dynamic equilibrium is set between IIR in the eluent and IIR adsorbed on the stationary phase.

The adsorbed IIR imparts a charge to the stationary phase so that it behaves as an ion-exchanger.

Total concentration of IIR adsorbed is dependent on percentage organic solvent in the mobile phase.

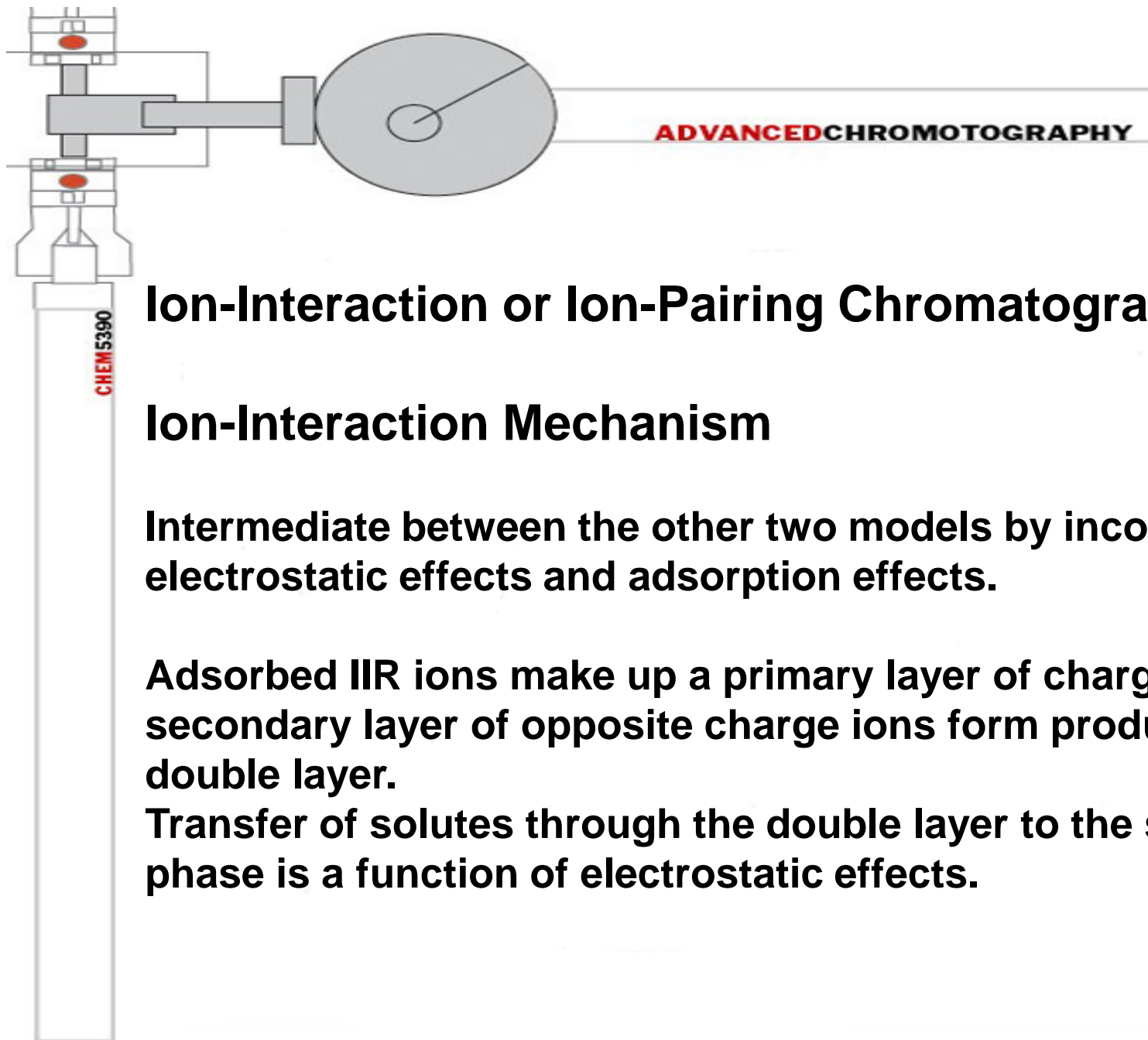
As organic solvent concentration increases IIR on stationary phase decreases.



Ion-Interaction or Ion-Pairing Chromatography

Dynamic Ion-Exchange Mechanism

Solutes having the same charge as the IIR are repelled from the charged stationary phase and have decrease retention times.



Ion-Interaction or Ion-Pairing Chromatography

Ion-Interaction Mechanism

Intermediate between the other two models by incorporating electrostatic effects and adsorption effects.

Adsorbed IIR ions make up a primary layer of charge, then a secondary layer of opposite charge ions form producing a double layer.

Transfer of solutes through the double layer to the stationary phase is a function of electrostatic effects.

Ion Chromatography

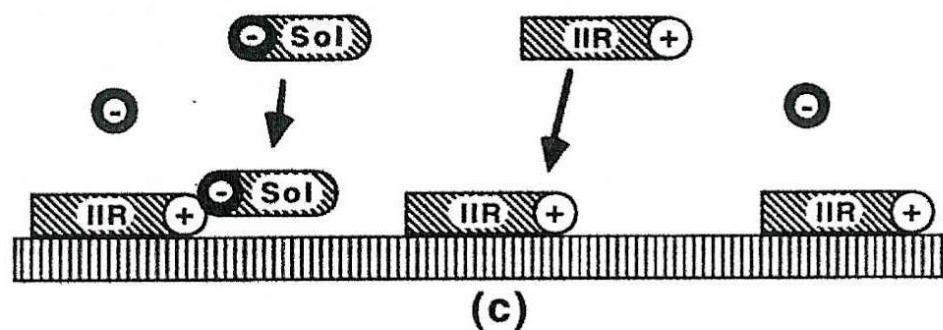
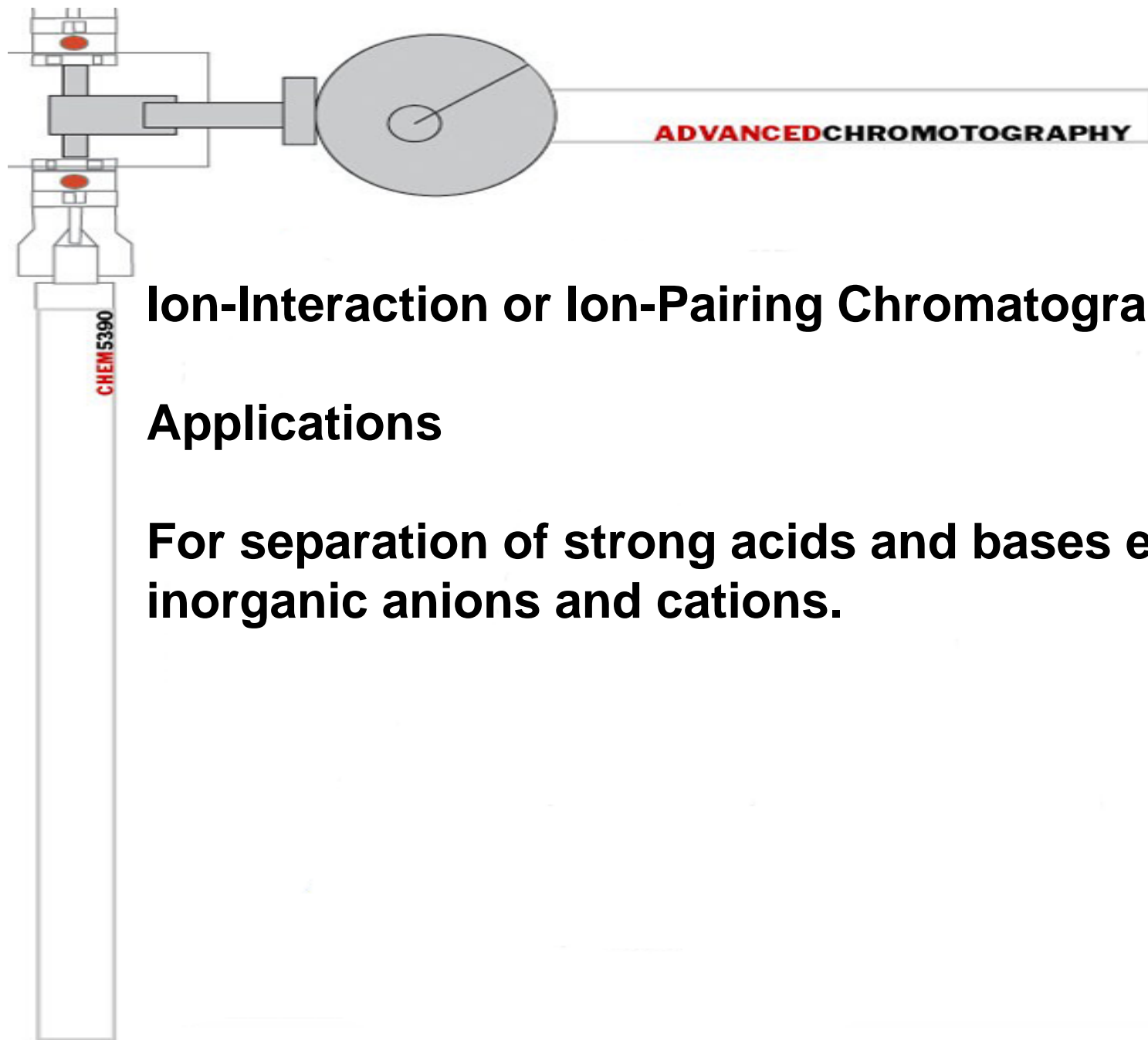


Fig. 6.29. Schematic illustration of (a) the ion-pair, (b) the dynamic ion-exchange and (c) the ion-interaction models for the retention of anionic solutes in the presence of a lipophilic cationic IIR. The solute and the IIR are labelled on the diagram. The large, hatched box represents the lipophilic stationary phase, the black circle with the negative charge represents the counter-anion of the IIR, while the white circle with the positive charge represents the counter-cation of the solute. Adapted from Bidlingmeyer (1980) *J. Chromatogr. Sci.*, **18**, 525.



Ion-Interaction or Ion-Pairing Chromatography

Applications

For separation of strong acids and bases especially inorganic anions and cations.

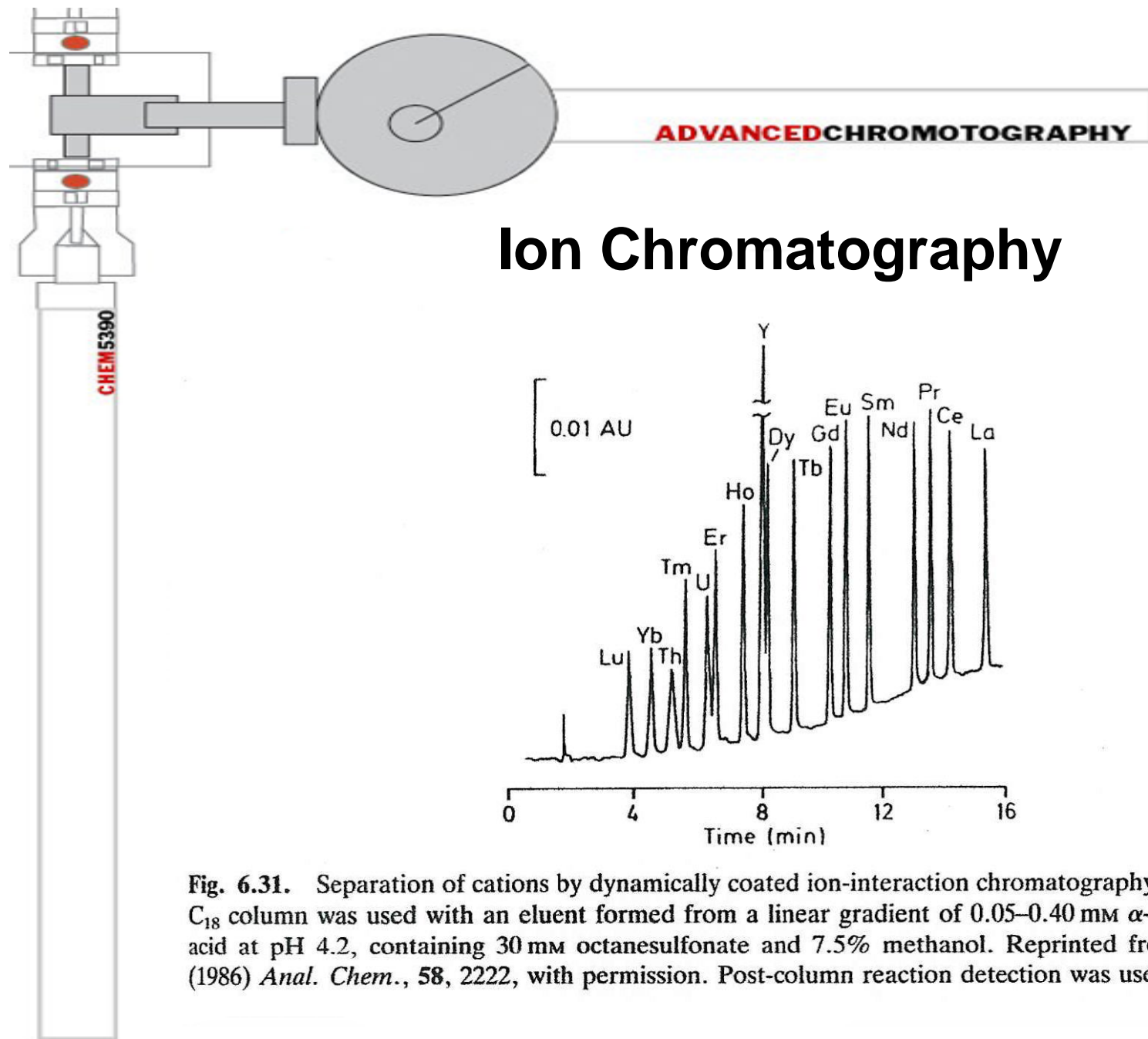
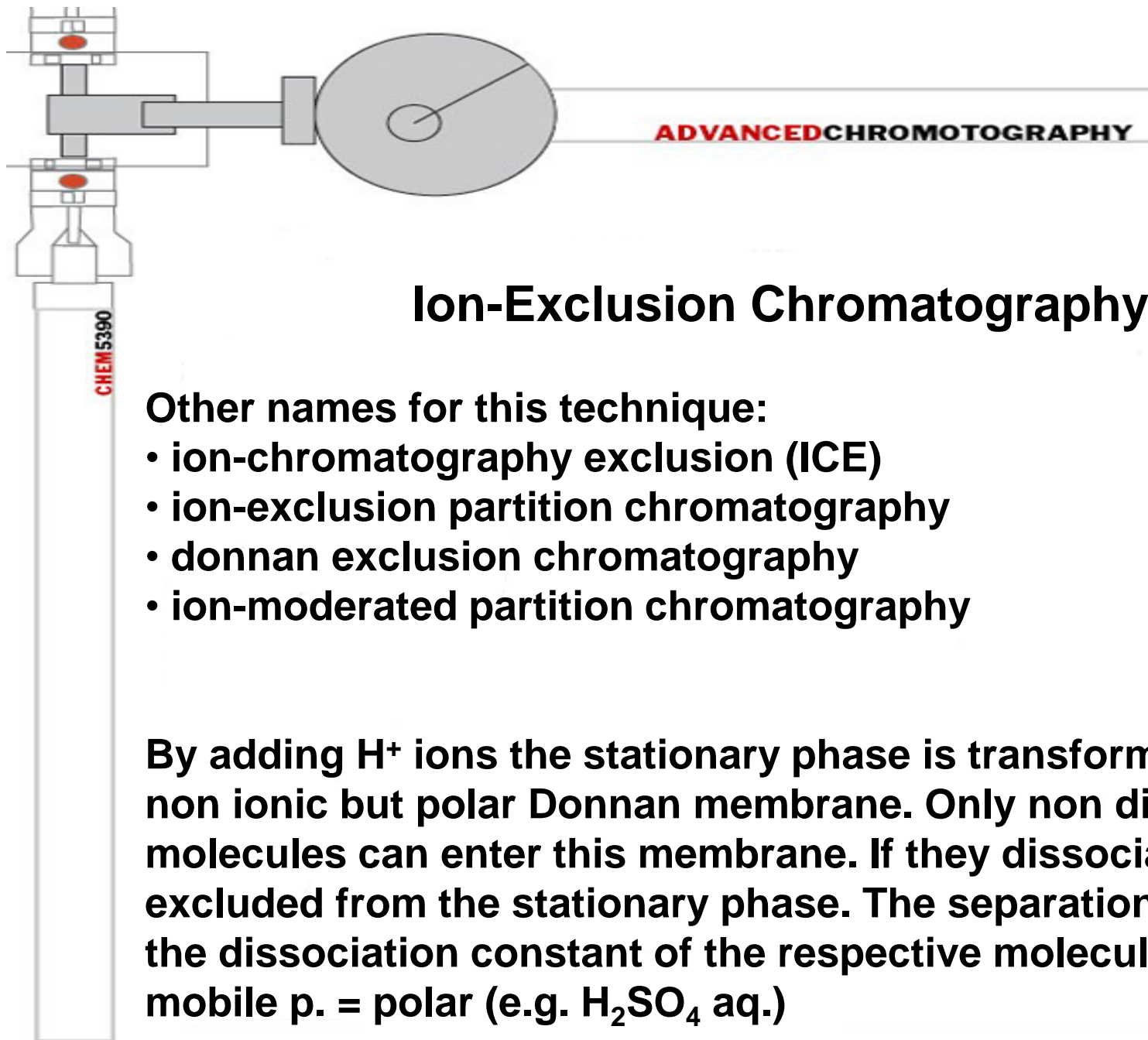


Fig. 6.31. Separation of cations by dynamically coated ion-interaction chromatography. A 5 μm Supelco C_{18} column was used with an eluent formed from a linear gradient of 0.05–0.40 mM α -hydroxyisobutyric acid at pH 4.2, containing 30 mM octanesulfonate and 7.5% methanol. Reprinted from Barkley *et al.* (1986) *Anal. Chem.*, **58**, 2222, with permission. Post-column reaction detection was used.

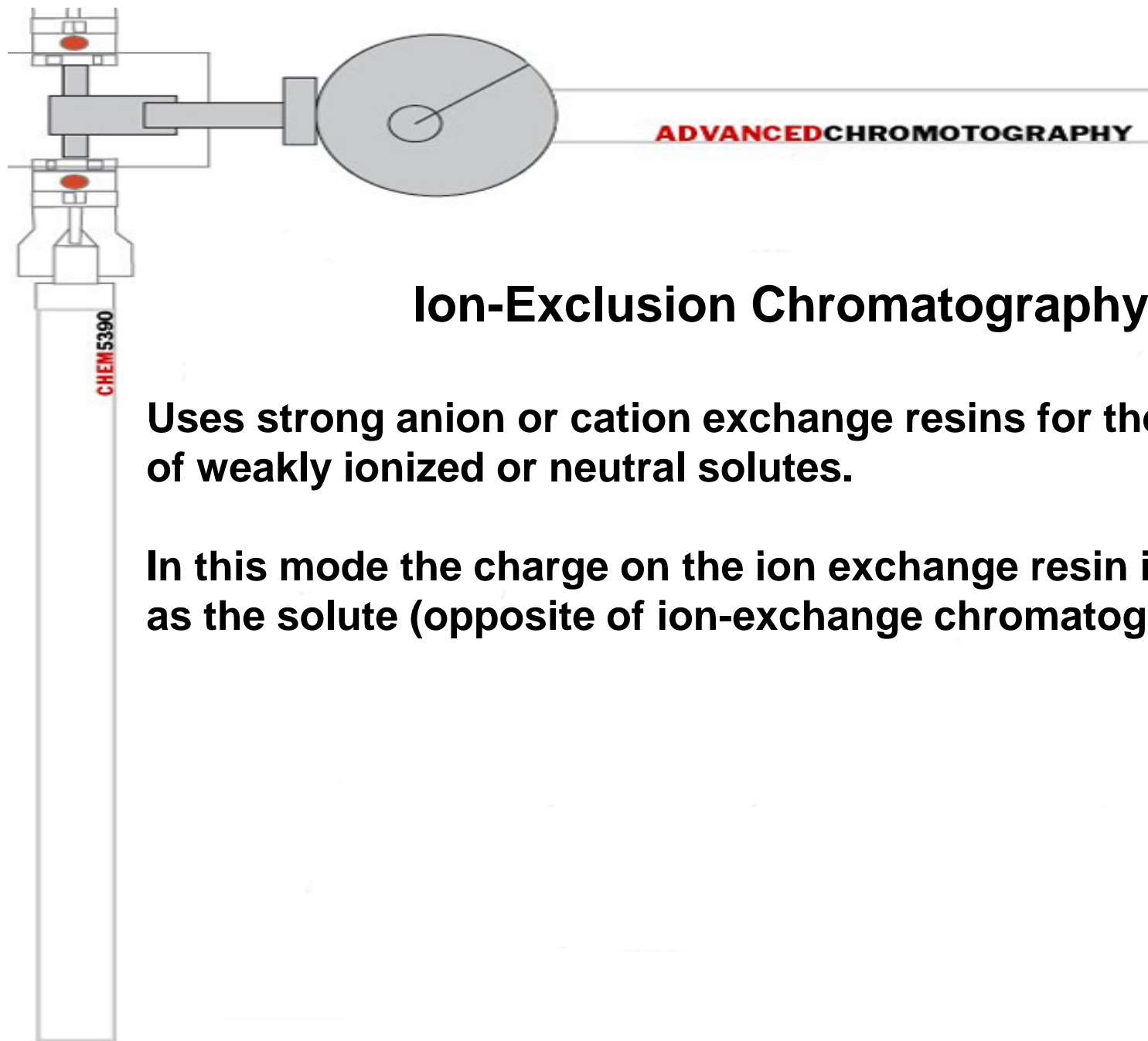


Ion-Exclusion Chromatography

Other names for this technique:

- **ion-chromatography exclusion (ICE)**
- **ion-exclusion partition chromatography**
- **donnan exclusion chromatography**
- **ion-moderated partition chromatography**

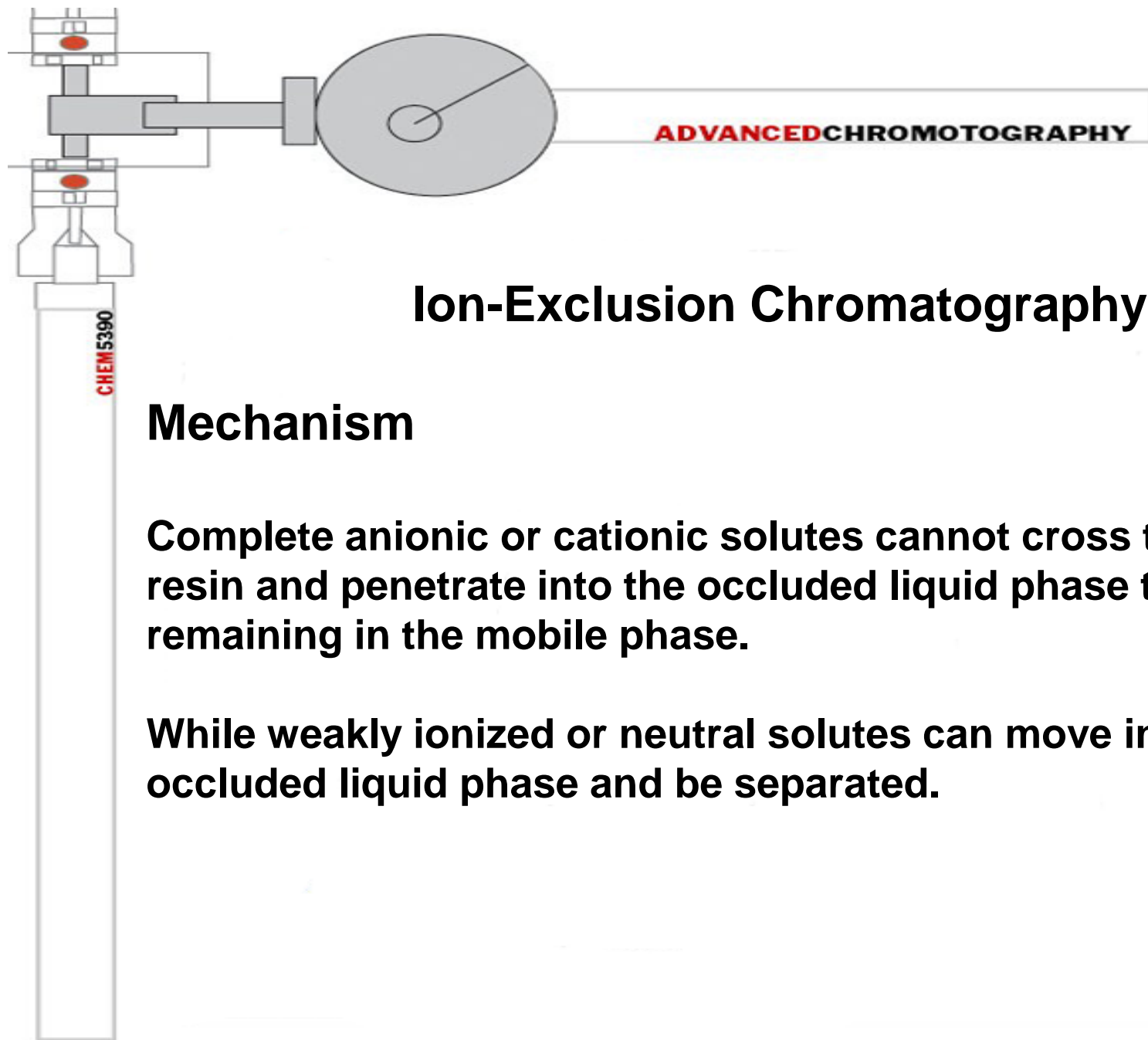
By adding H^+ ions the stationary phase is transformed into a non ionic but polar Donnan membrane. Only non dissociated molecules can enter this membrane. If they dissociate they are excluded from the stationary phase. The separation depends on the dissociation constant of the respective molecules.
mobile p. = polar (e.g. H_2SO_4 aq.)



Ion-Exclusion Chromatography

Uses strong anion or cation exchange resins for the separation of weakly ionized or neutral solutes.

In this mode the charge on the ion exchange resin is the same as the solute (opposite of ion-exchange chromatography).



Ion-Exclusion Chromatography

Mechanism

Complete anionic or cationic solutes cannot cross the ionized resin and penetrate into the occluded liquid phase thus remaining in the mobile phase.

While weakly ionized or neutral solutes can move into the occluded liquid phase and be separated.

Ion-Exclusion Chromatography

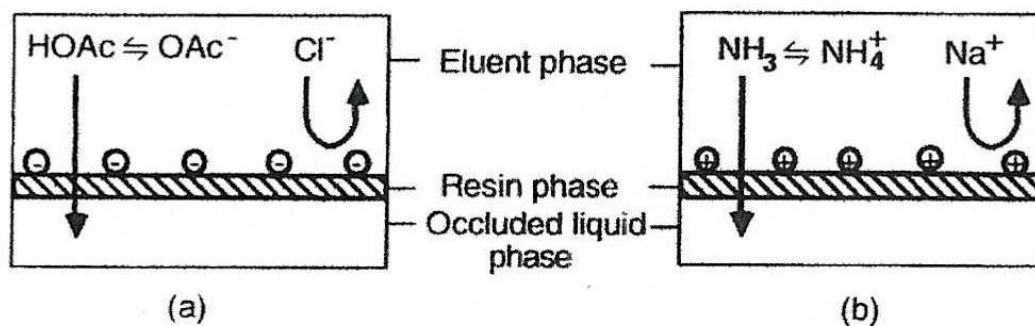
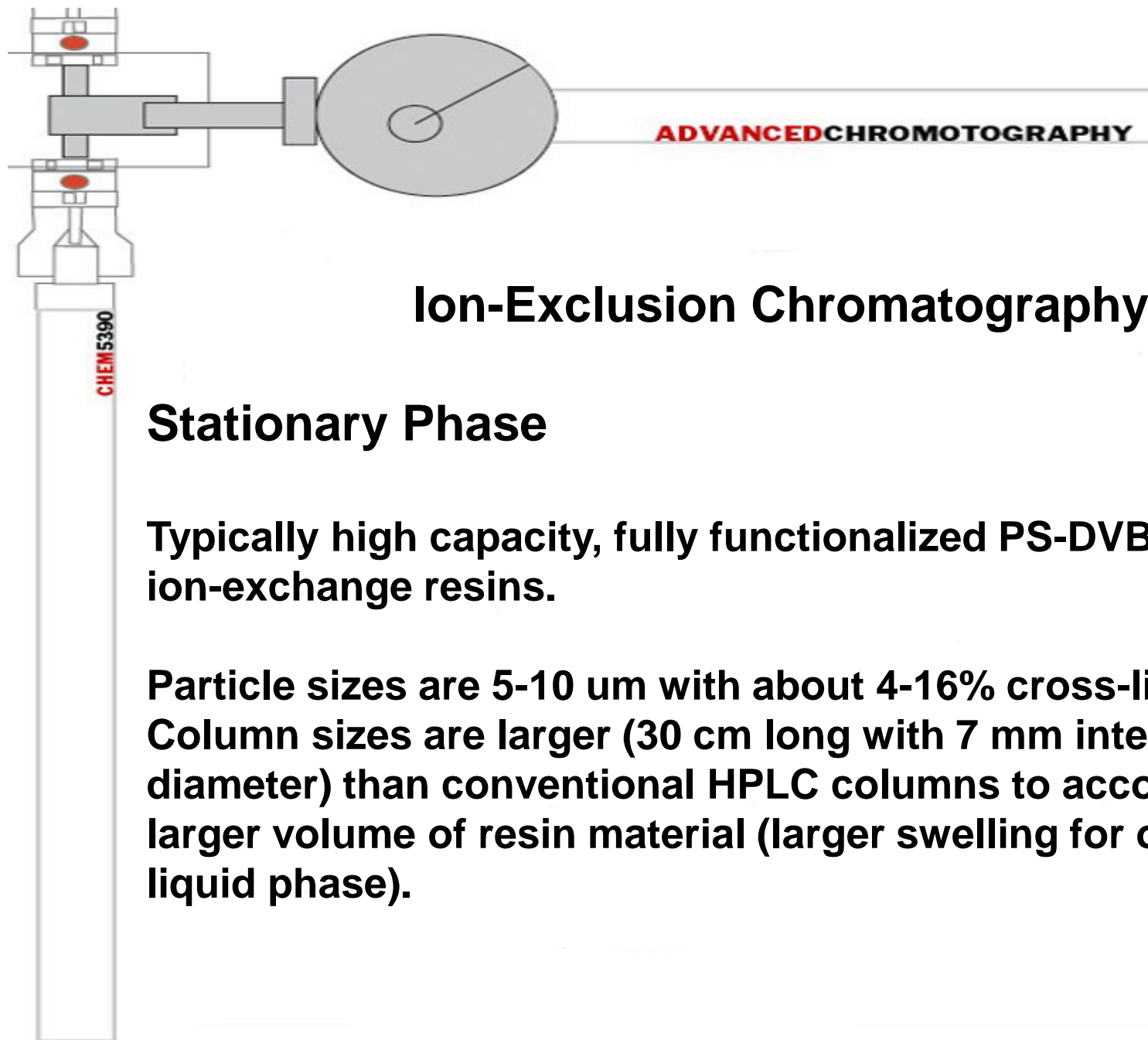


Fig. 6.40. Schematic representation of ion-exclusion chromatography for (a) acidic solutes, such as acetic acid and HCl, and (b) basic solutes, such as NH_3 and NaOH.

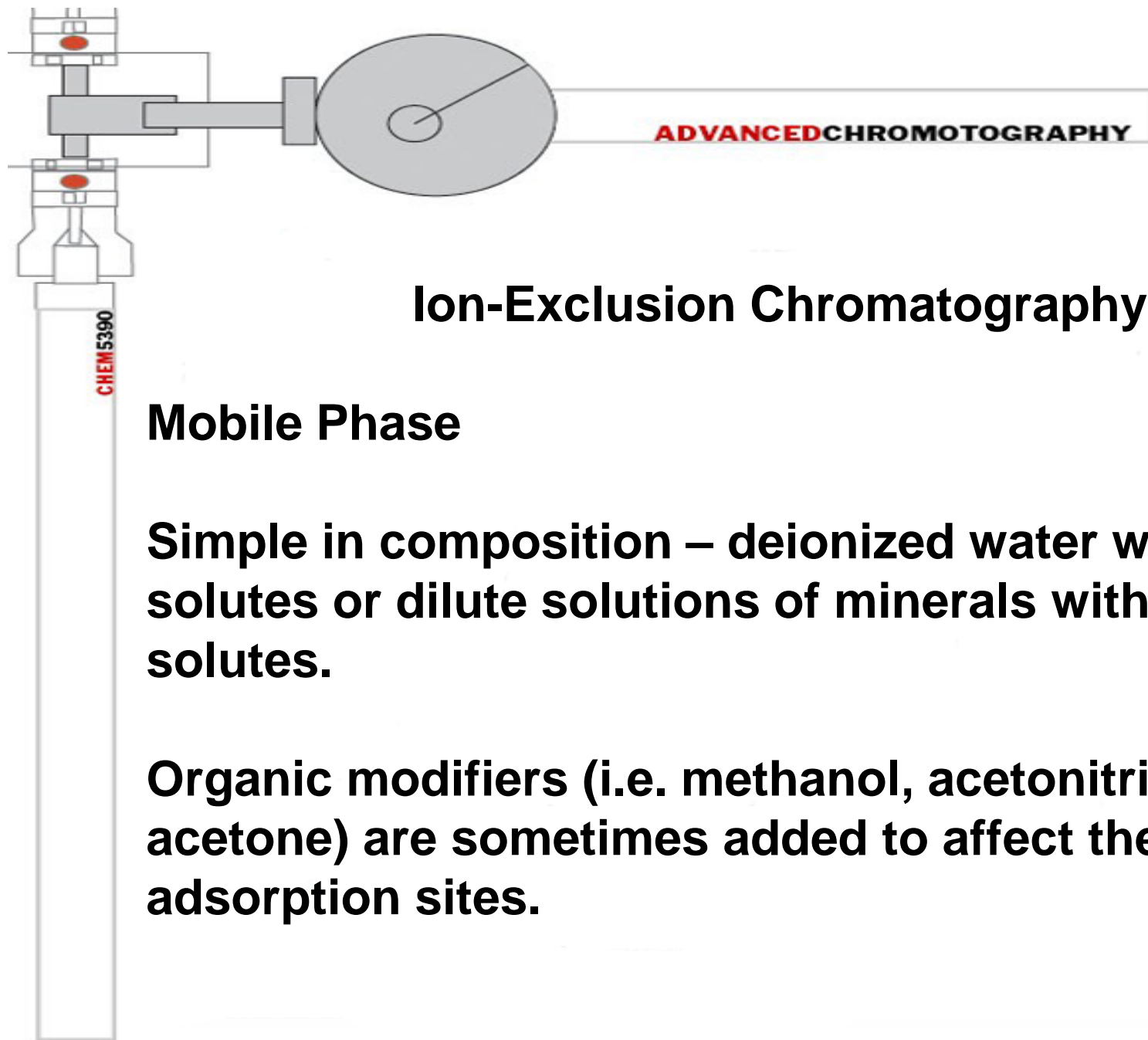


Ion-Exclusion Chromatography

Stationary Phase

Typically high capacity, fully functionalized PS-DVB polymeric ion-exchange resins.

Particle sizes are 5-10 μm with about 4-16% cross-linking. Column sizes are larger (30 cm long with 7 mm internal diameter) than conventional HPLC columns to accommodate the larger volume of resin material (larger swelling for occluded liquid phase).

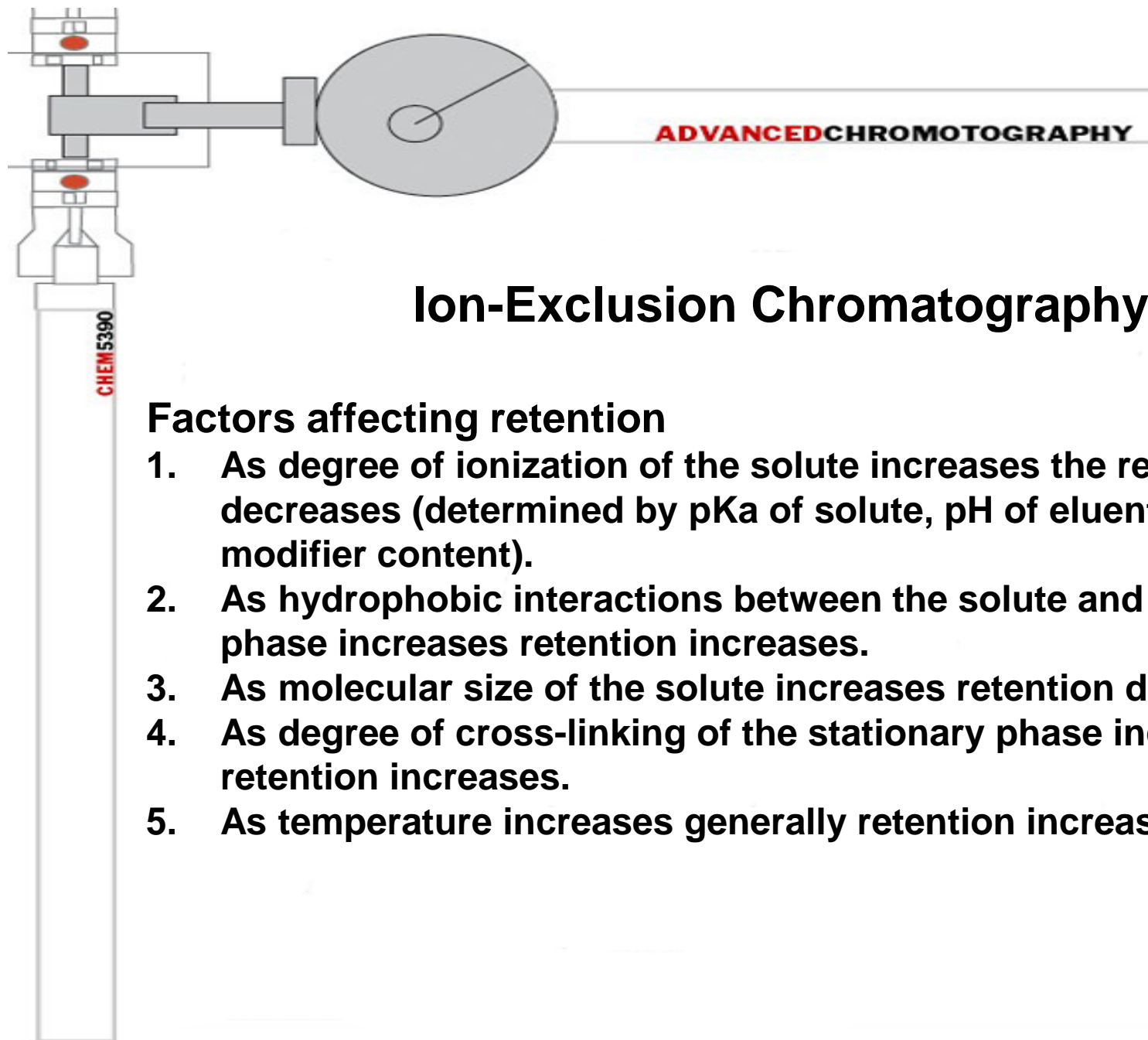


Ion-Exclusion Chromatography

Mobile Phase

Simple in composition – deionized water with the solutes or dilute solutions of minerals with the solutes.

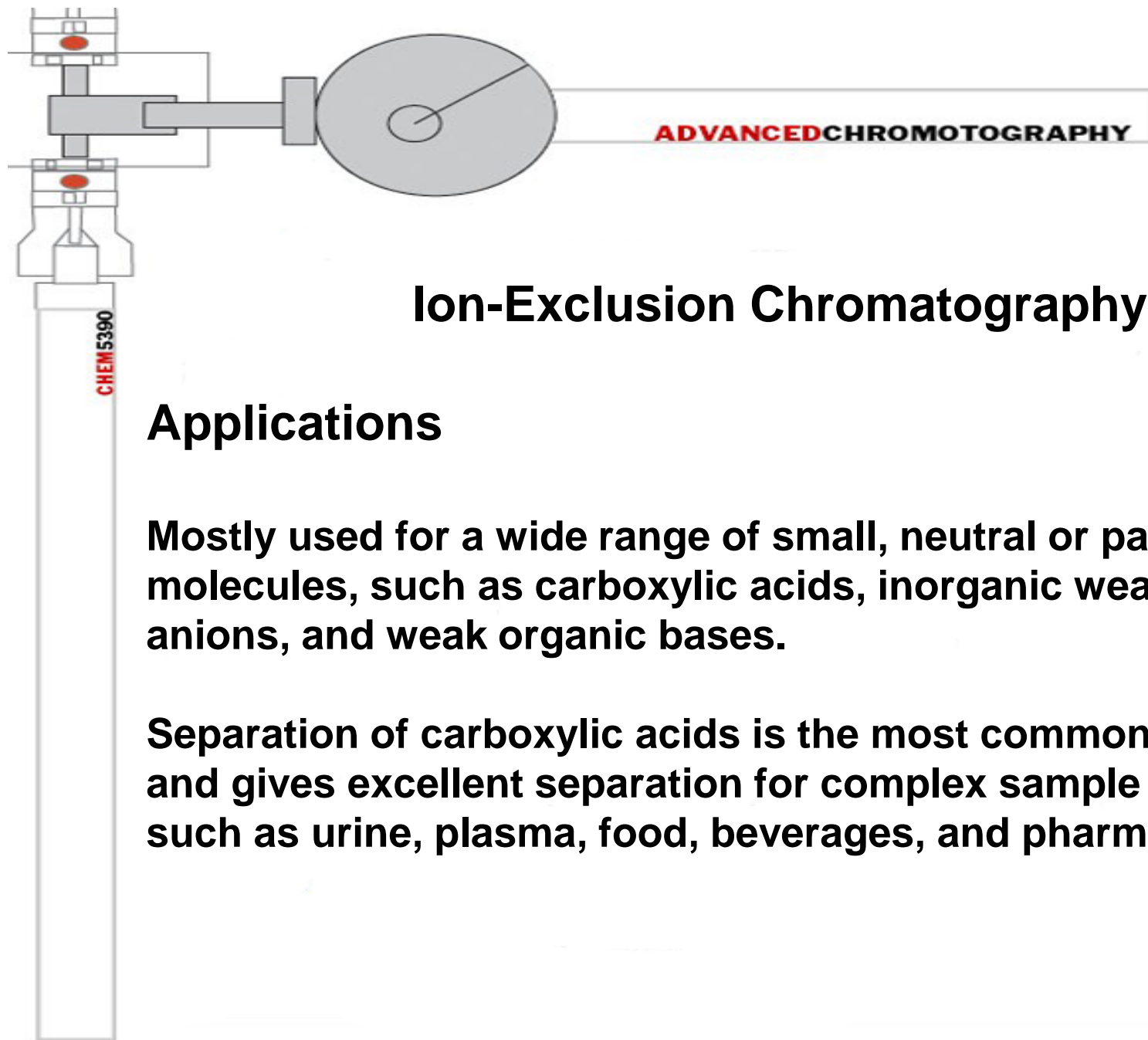
Organic modifiers (i.e. methanol, acetonitrile, acetone) are sometimes added to affect the adsorption sites.



Ion-Exclusion Chromatography

Factors affecting retention

- 1. As degree of ionization of the solute increases the retention decreases (determined by pKa of solute, pH of eluent and organic modifier content).**
- 2. As hydrophobic interactions between the solute and the stationary phase increases retention increases.**
- 3. As molecular size of the solute increases retention decreases.**
- 4. As degree of cross-linking of the stationary phase increases retention increases.**
- 5. As temperature increases generally retention increases.**



Ion-Exclusion Chromatography

Applications

Mostly used for a wide range of small, neutral or partially ionized molecules, such as carboxylic acids, inorganic weak acid anions, and weak organic bases.

Separation of carboxylic acids is the most common application and gives excellent separation for complex sample matrices, such as urine, plasma, food, beverages, and pharmaceuticals.

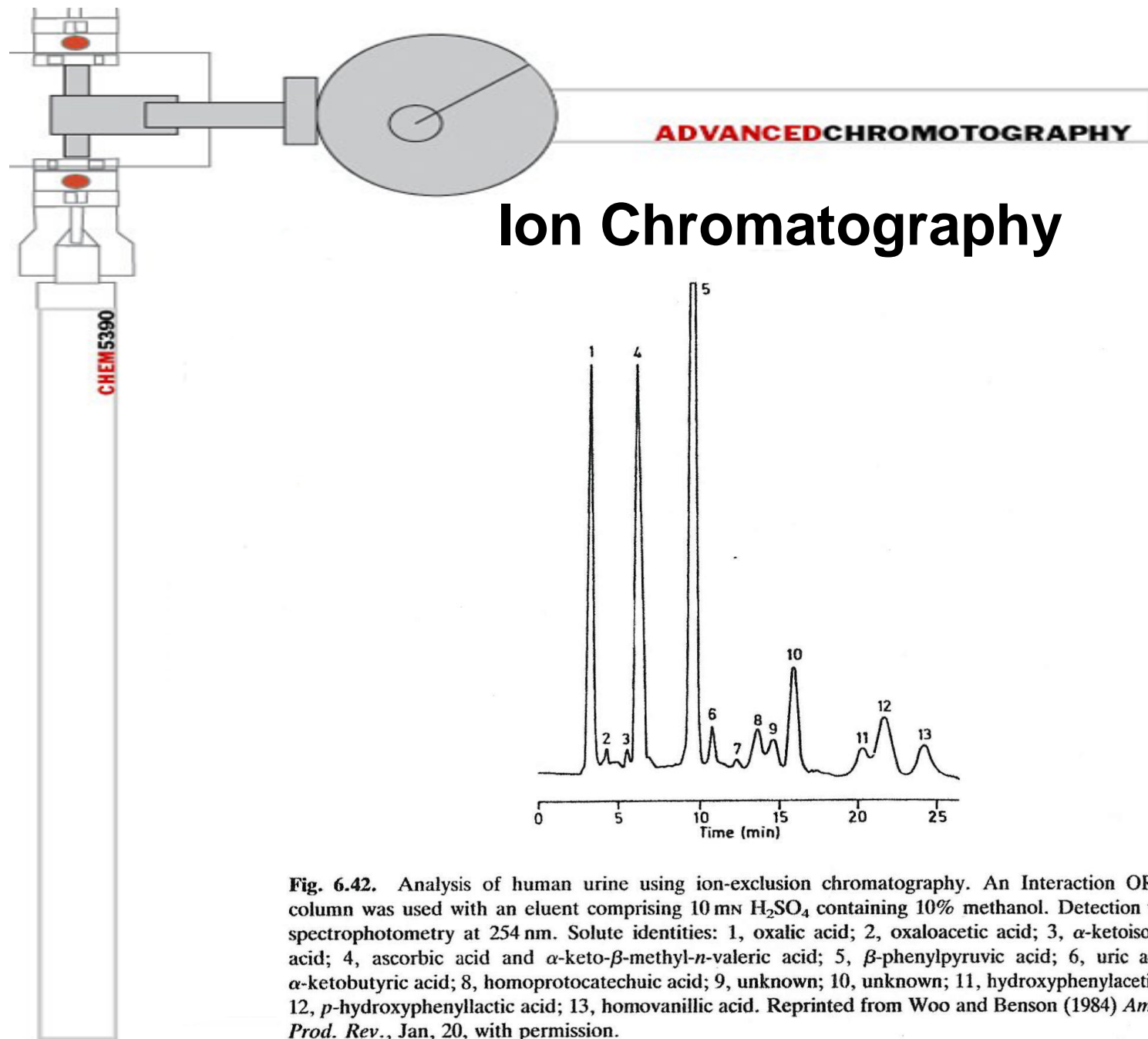
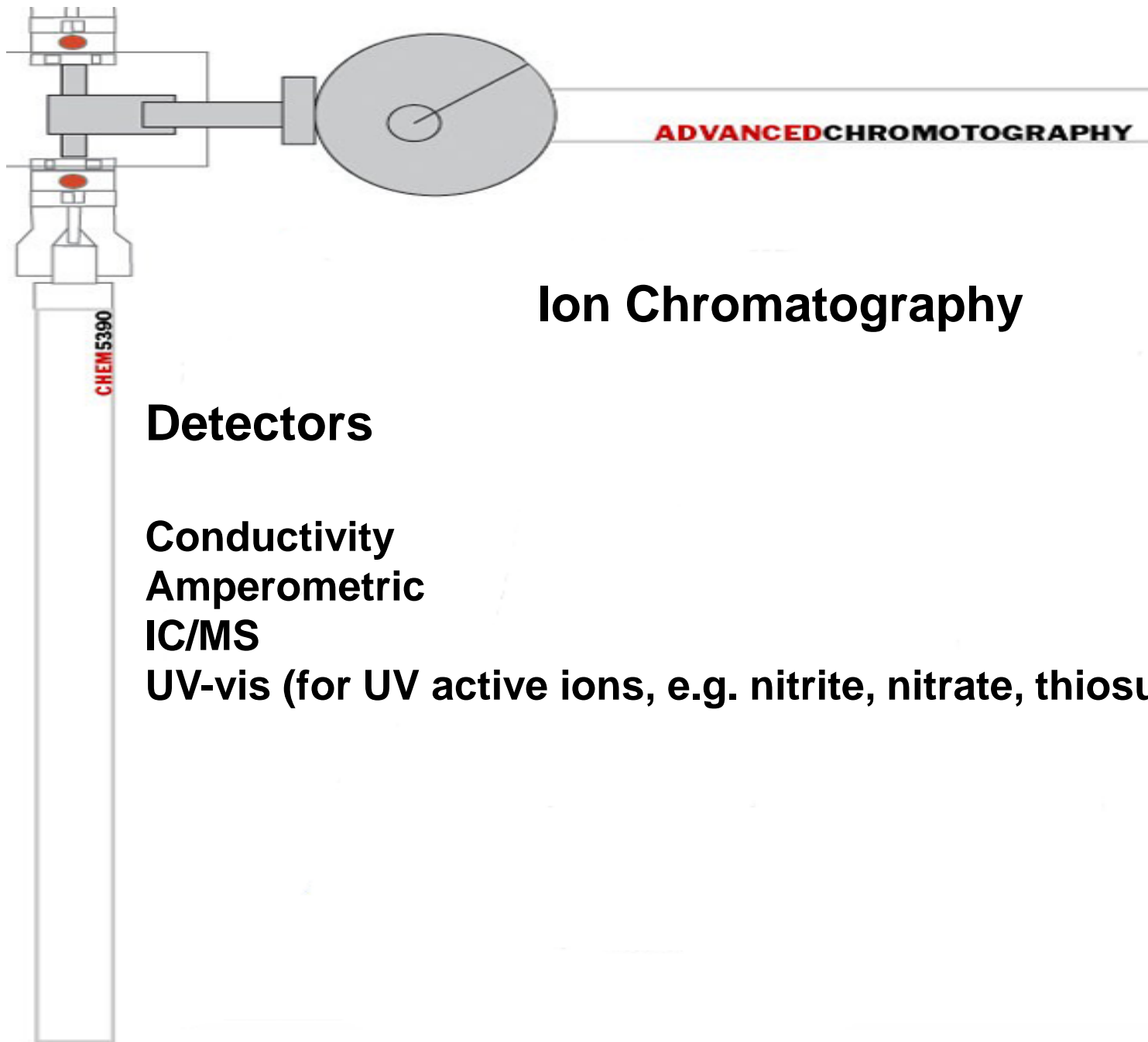


Fig. 6.42. Analysis of human urine using ion-exclusion chromatography. An Interaction ORH-801 column was used with an eluent comprising 10 mM H_2SO_4 containing 10% methanol. Detection was by spectrophotometry at 254 nm. Solute identities: 1, oxalic acid; 2, oxaloacetic acid; 3, α -ketoisovaleric acid; 4, ascorbic acid and α -keto- β -methyl-*n*-valeric acid; 5, β -phenylpyruvic acid; 6, uric acid; 7, α -ketobutyric acid; 8, homoprotocatechuic acid; 9, unknown; 10, unknown; 11, hydroxyphenylacetic acid; 12, *p*-hydroxyphenyllactic acid; 13, homovanillic acid. Reprinted from Woo and Benson (1984) *Am. Clin. Prod. Rev.*, Jan, 20, with permission.



Ion Chromatography

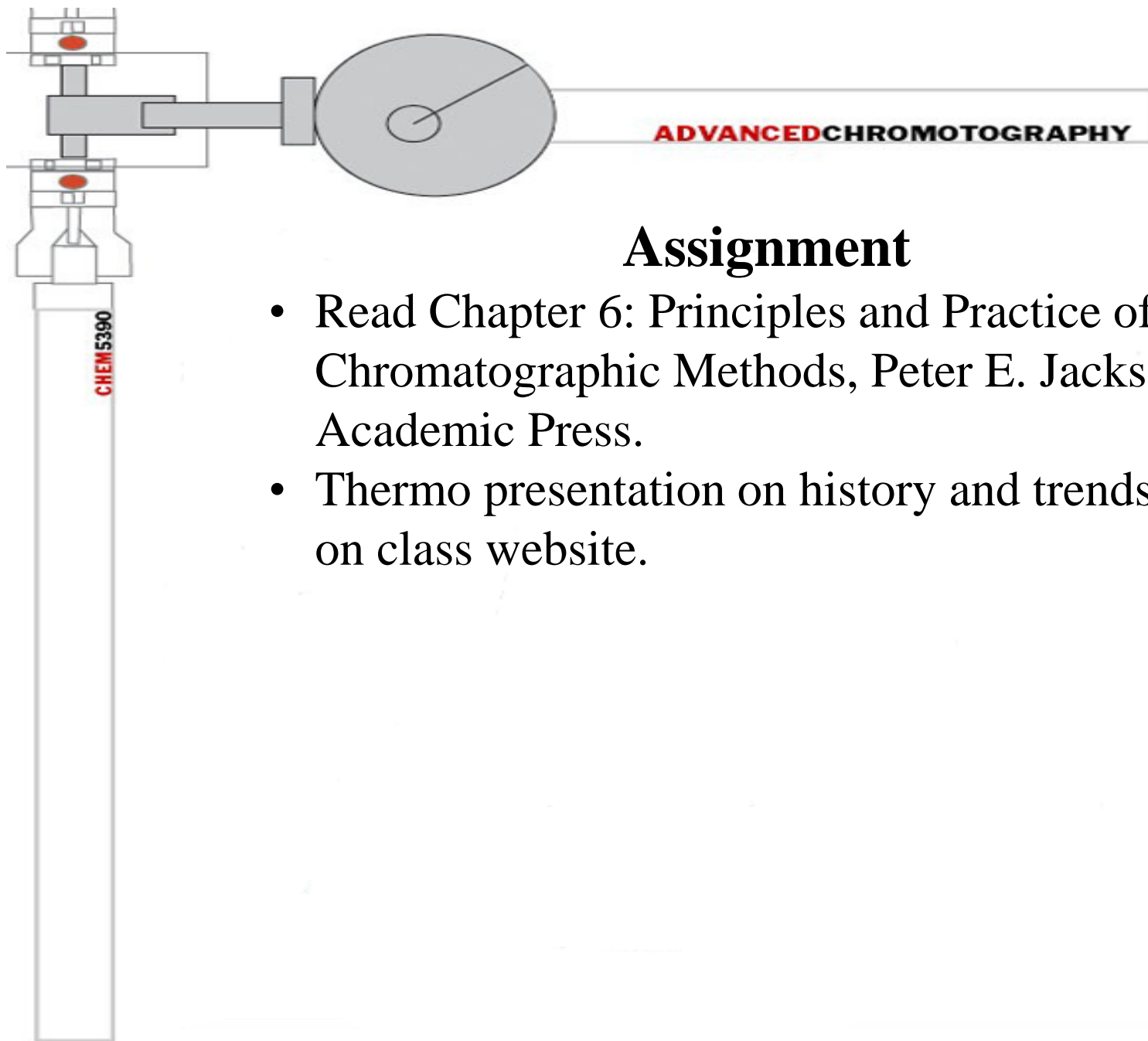
Detectors

Conductivity

Amperometric

IC/MS

UV-vis (for UV active ions, e.g. nitrite, nitrate, thiosulfate)



Assignment

- Read Chapter 6: Principles and Practice of Modern Chromatographic Methods, Peter E. Jackson, Academic Press.
- Thermo presentation on history and trends of IC – on class website.