

System Peaks in Liquid Chromatography

Dr. Shulamit Levin
Medtechnica

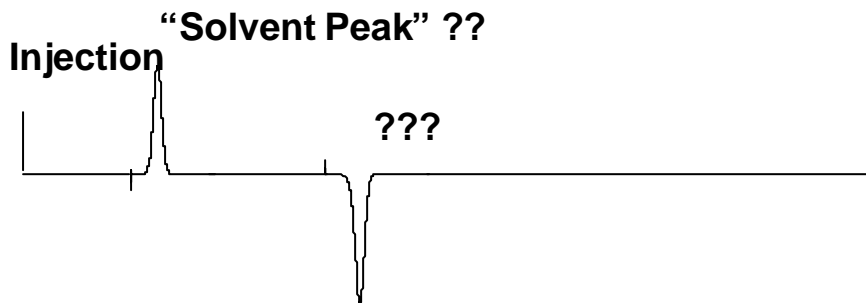
Tel: 052-448632; Fax: 03-9249977

Email: levins@medtechnica.net.il

Shulal@zahav.net.il

Home page: www.forumsci.co.il/HPLC

INJECTION OF PURE SOLVENT



Separation of Free Amino Acids by RP HPLC

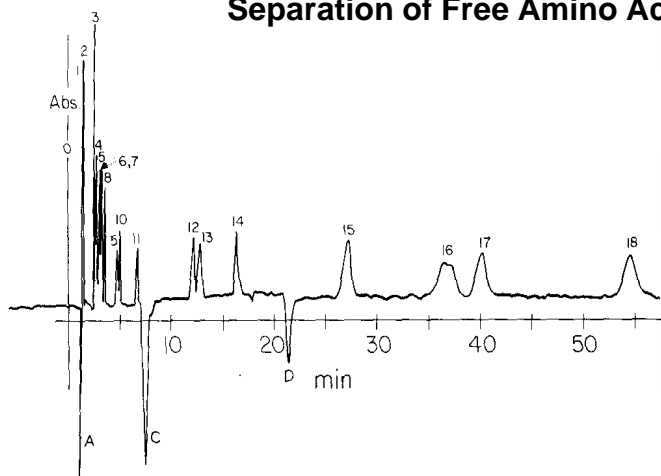


Fig. 7. Chromatogram obtained at 40°C. Mobile phase, 10 mM acetate buffer-0.4 mM copper(II) acetate-0.8 mM heptanesulfonate. Other conditions as in Fig. 1. Peaks: 1 = Asp; 2 = Glu; 3 = Gly + Ser; 4 = Asn; 5 = Gln; 6 = Thr; 7 = Ala; 8 = Thr; 9 = α Abu; 10 = His; 11 = Pro; 12 = Val; 13 = β Nvl; 14 = Met; 15 = Tyr; 16 = Ile; 17 = Leu; 18 = Arg.

Separation of Free Amino Acids by RP HPLC

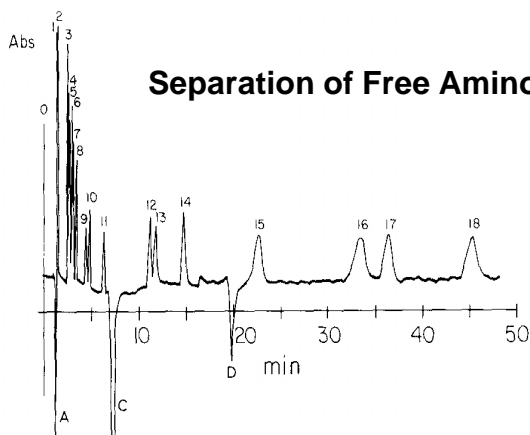
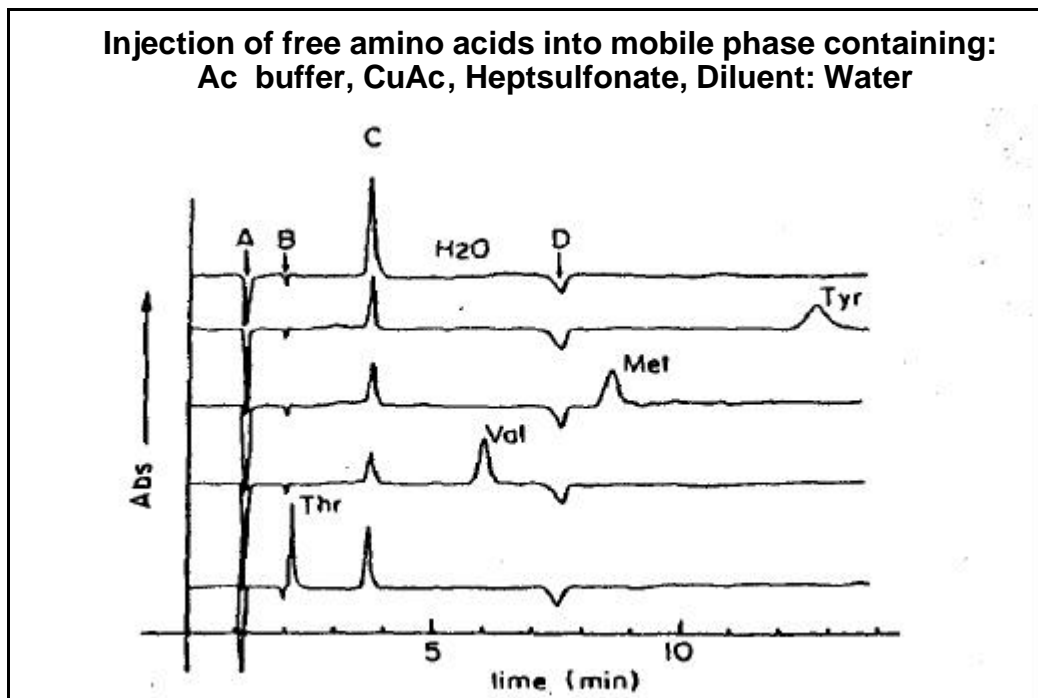
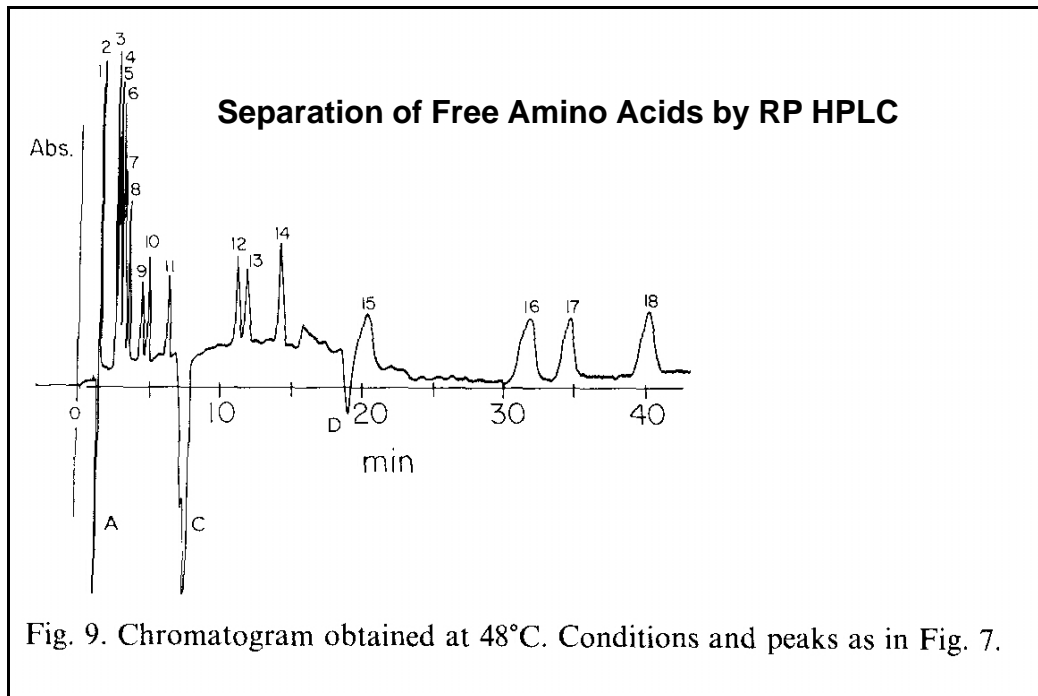
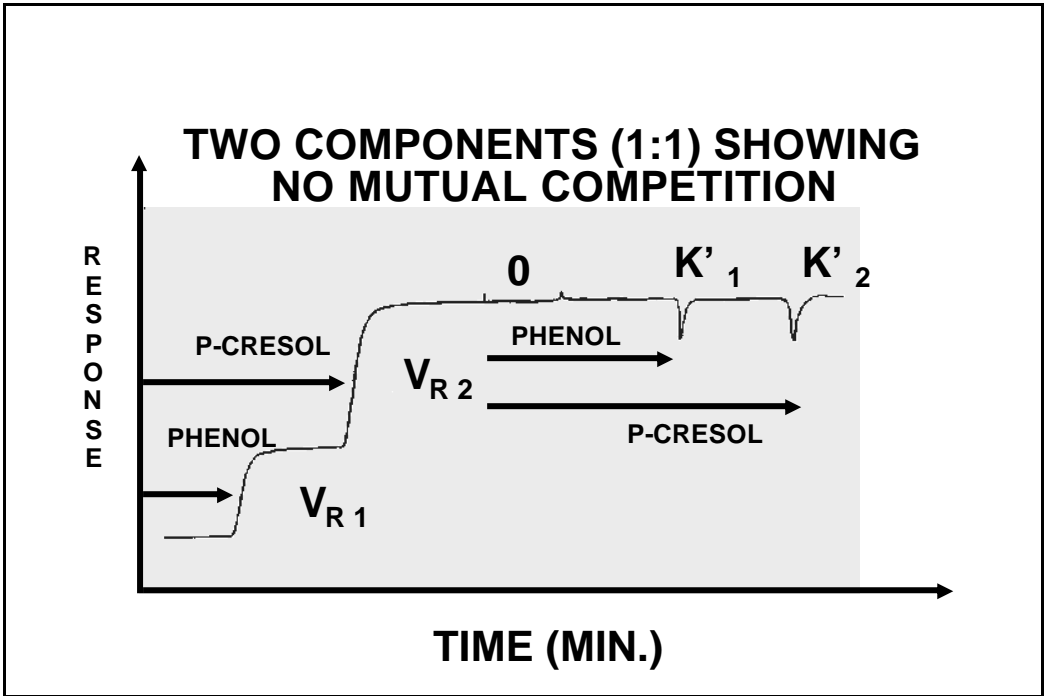
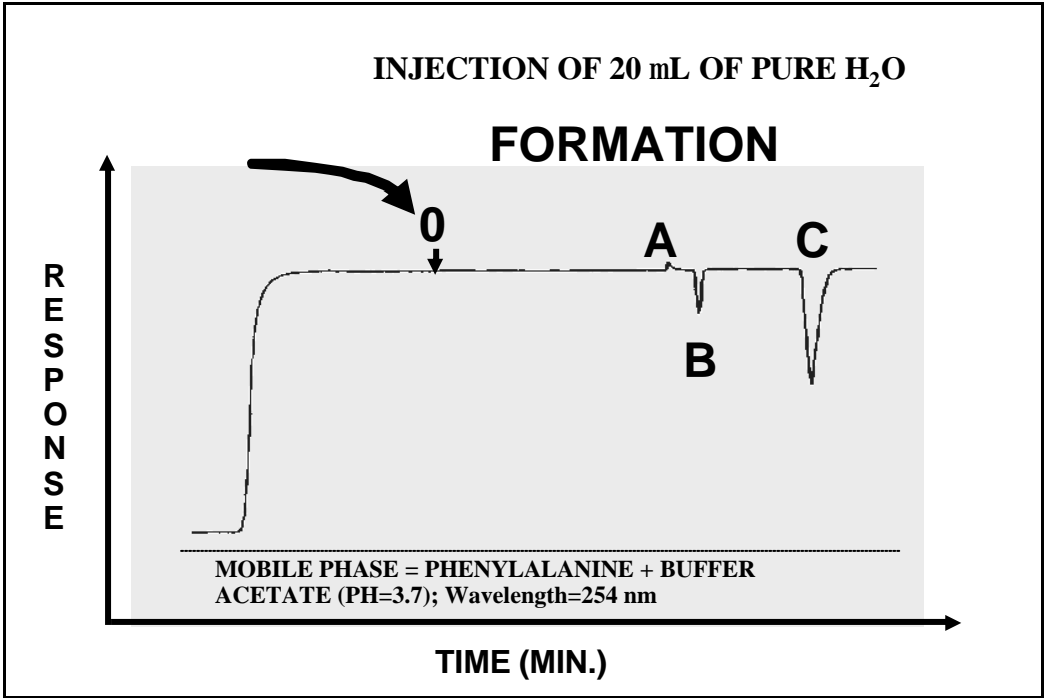
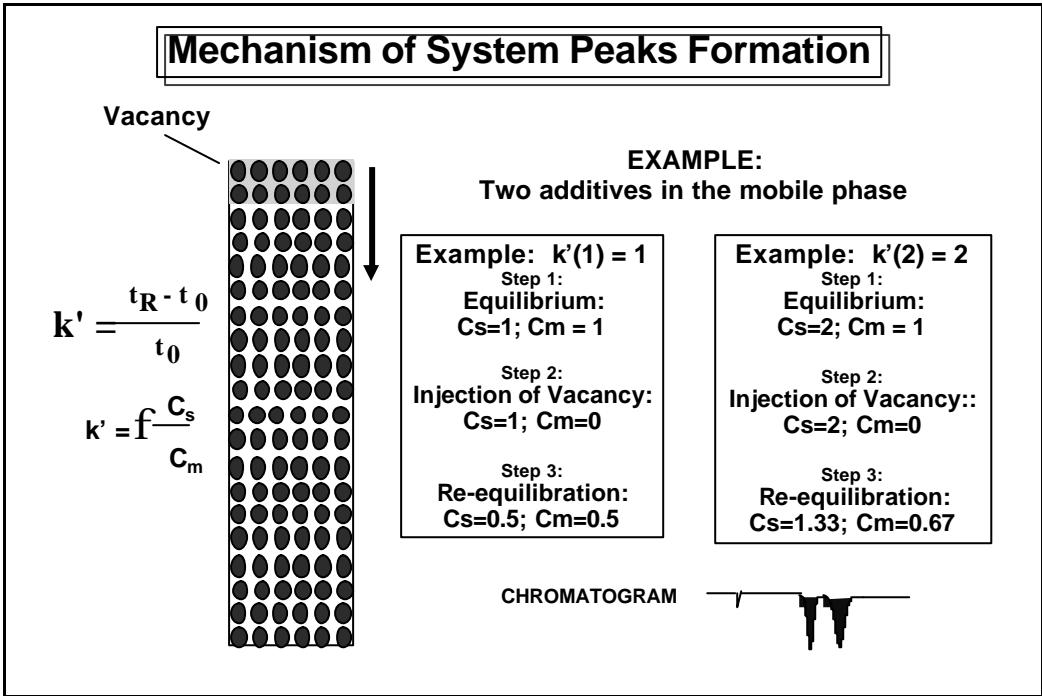
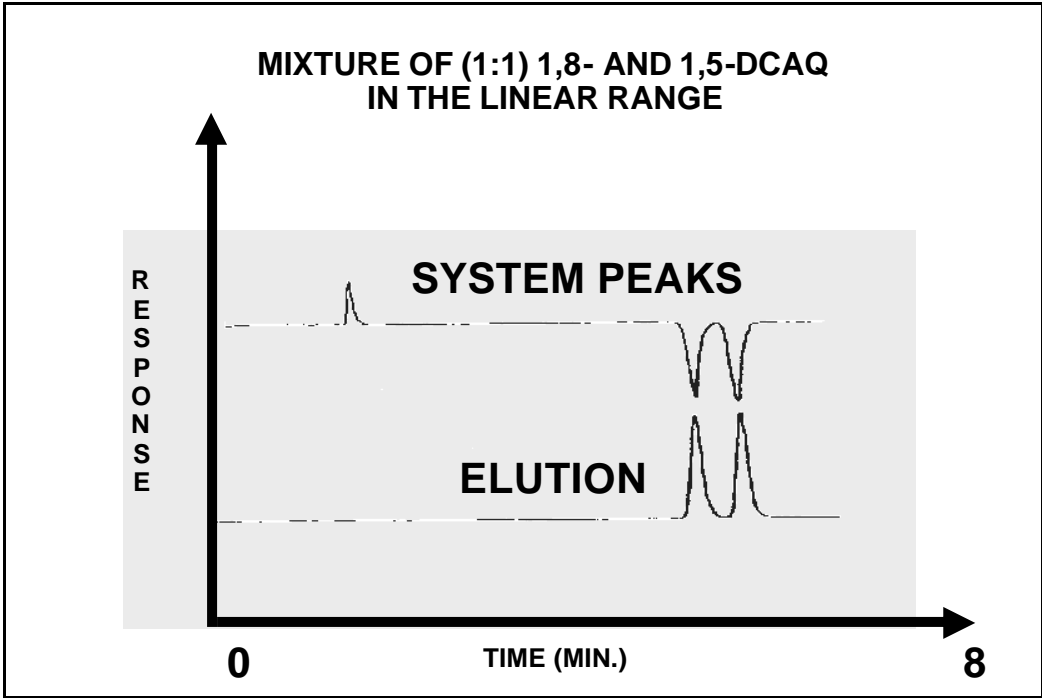


Fig. 8. Chromatogram obtained at 45°C. Conditions and peaks as in Fig. 7.

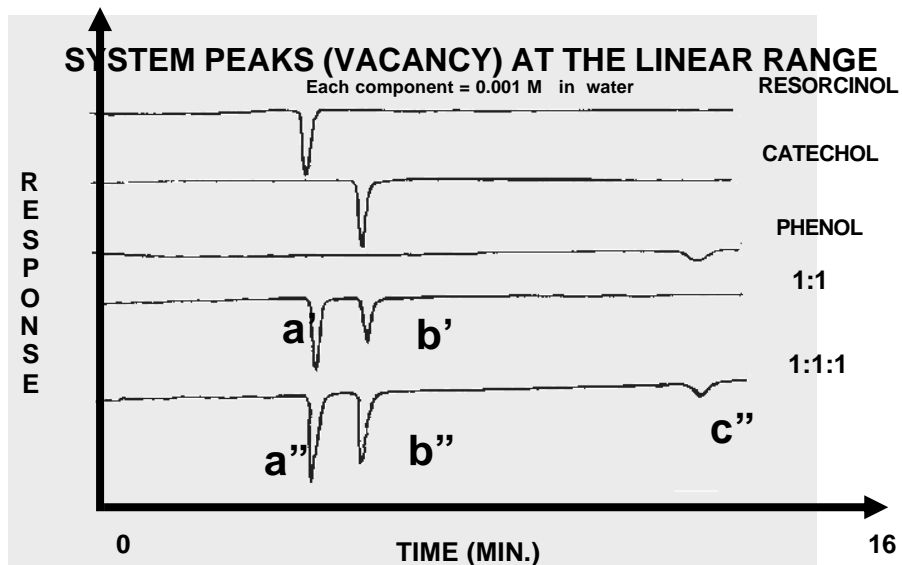




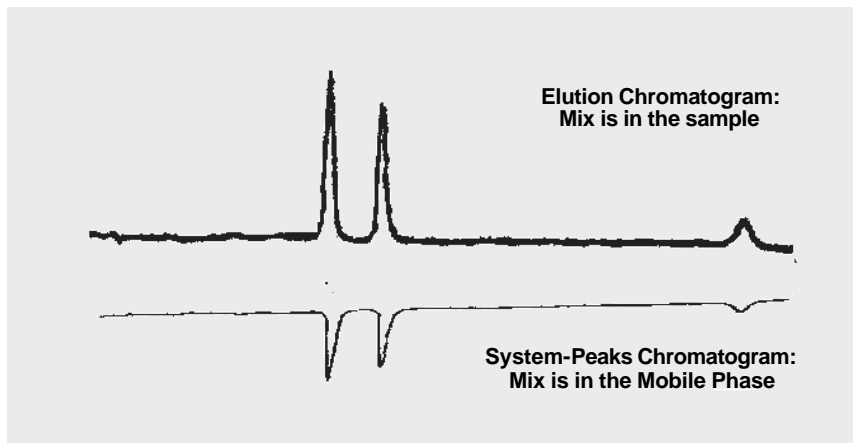


CONDITIONS FOR APPEARANCE OF SYSTEM PEAKS

- Mobile phase is multicomponent ($n \geq 2$)
- Mobile phase contains adsorbable components
- Mobile phase's components respond to the detector (high background)
- Sample or sample diluent are different than the mobile phase, enough to create equilibrium perturbation.

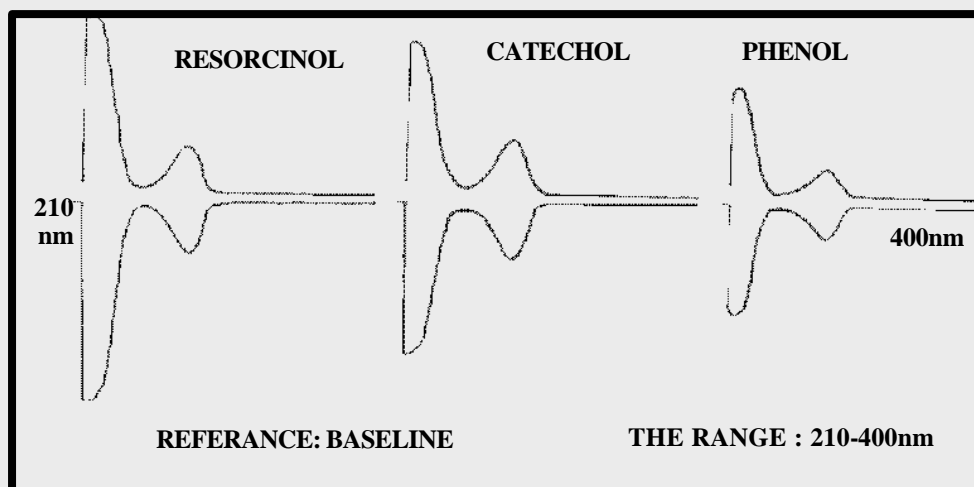


Resorcinol-Catechol-Phenol 1:1:1 Mixture in the Linear Range

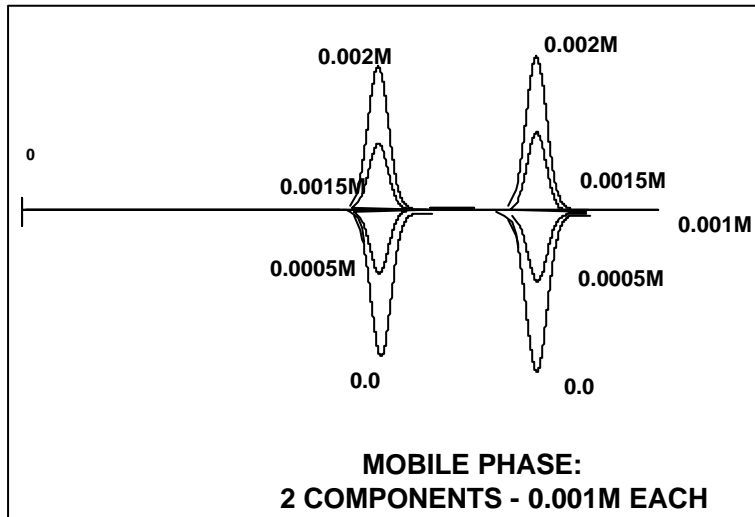


UV-SPECTRUM OF (1:1:1) MIXTURE

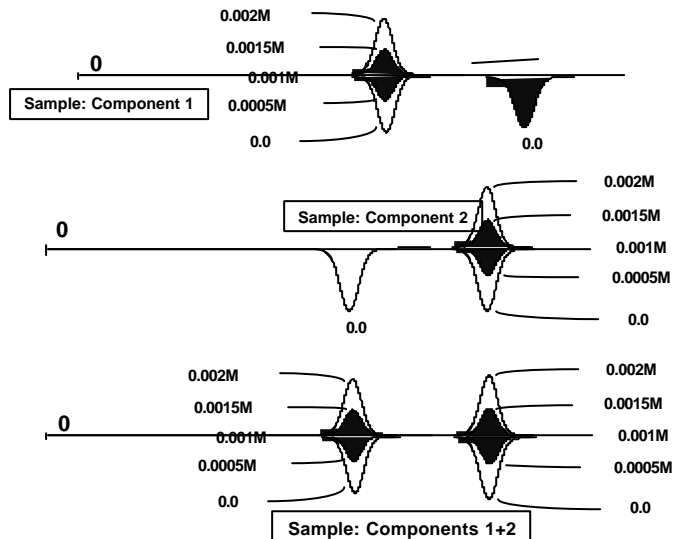
Apex of elution peak vs apex of system peak

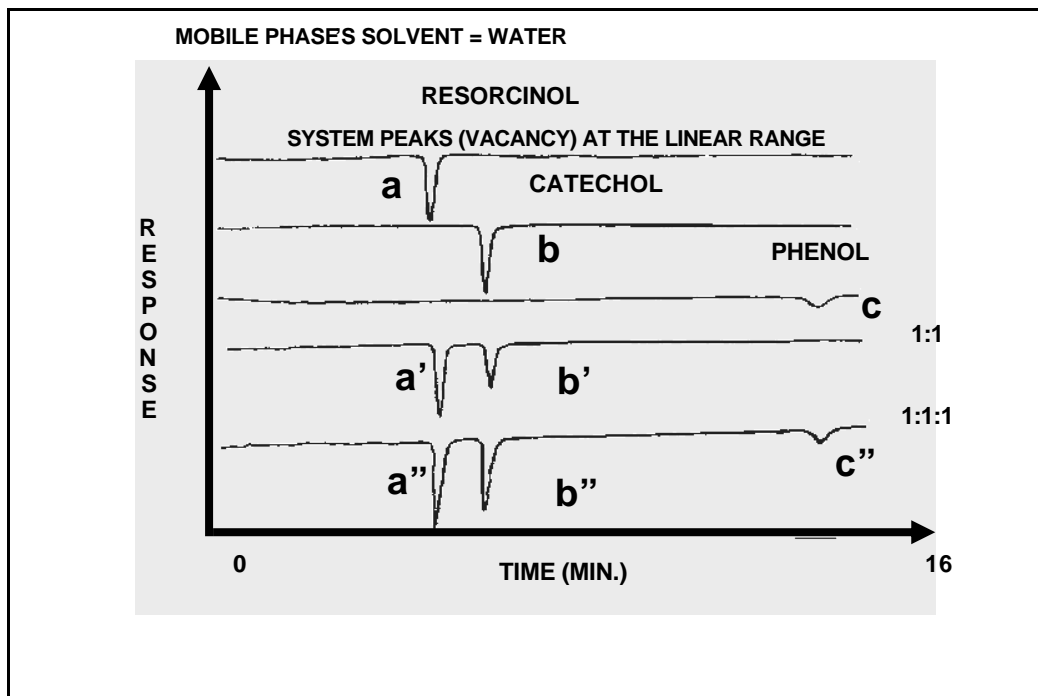
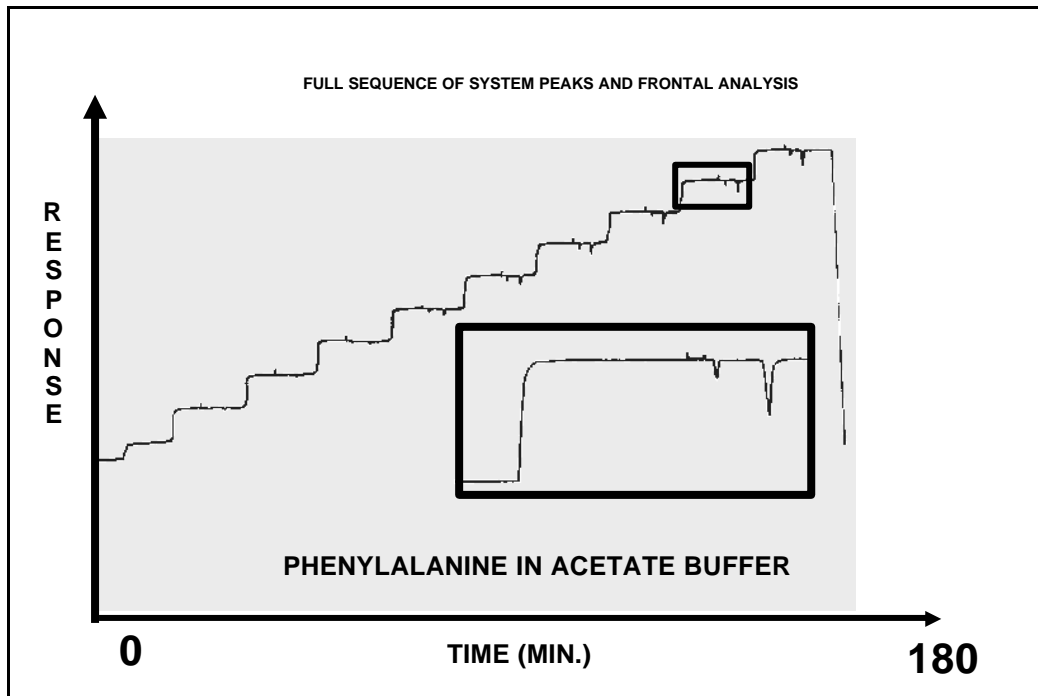


Detection of System Peaks

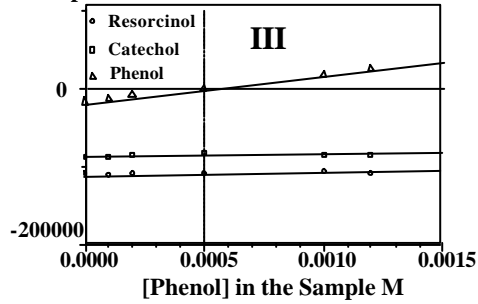
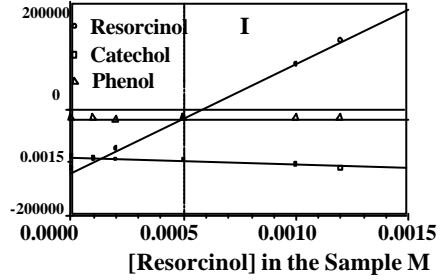
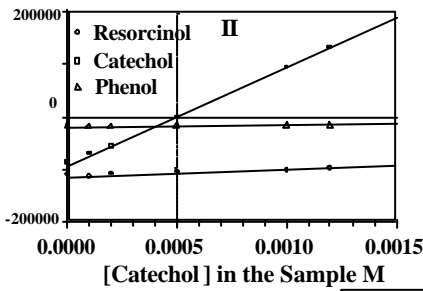


INJECTING MOBILE PHASE COMPONENTS : VARIOUS CONCENTRATIONS IN THE SAMPLE

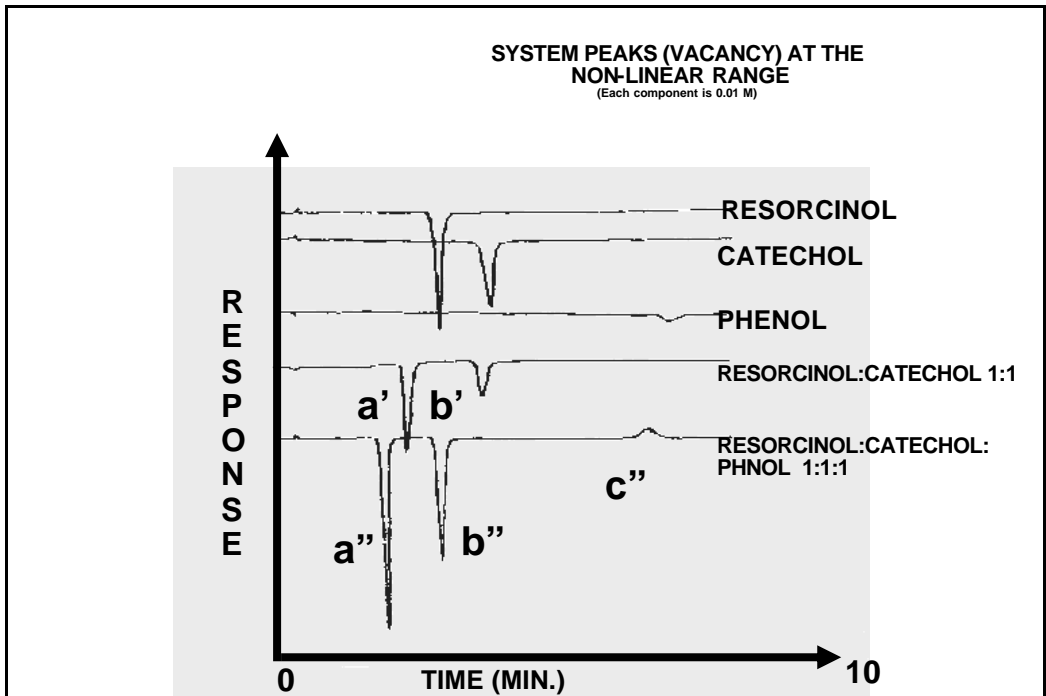
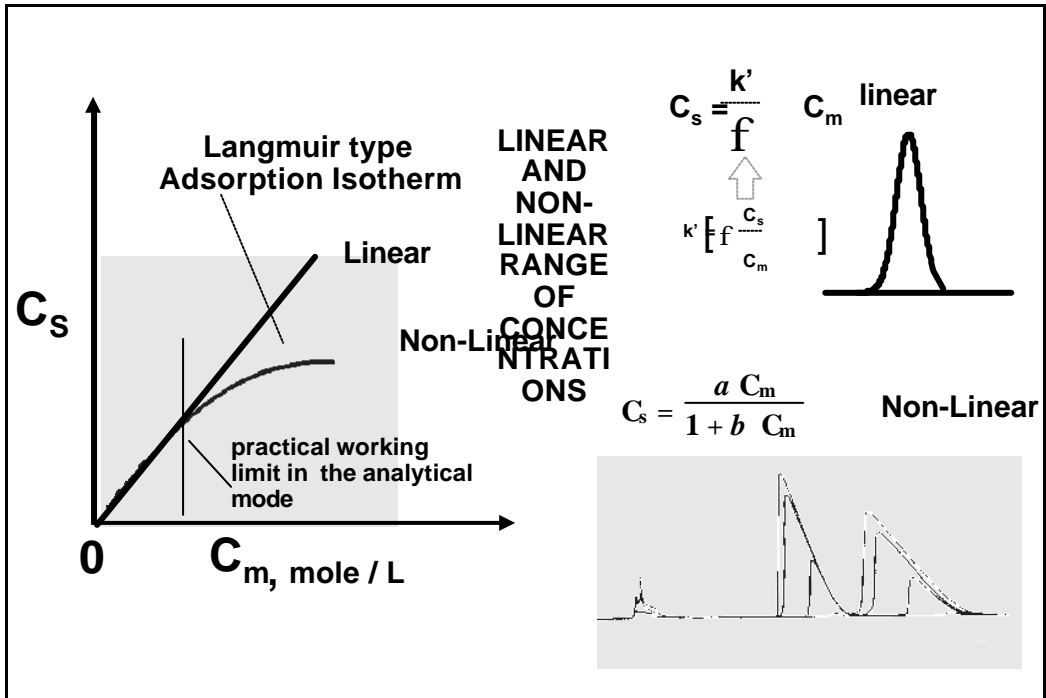




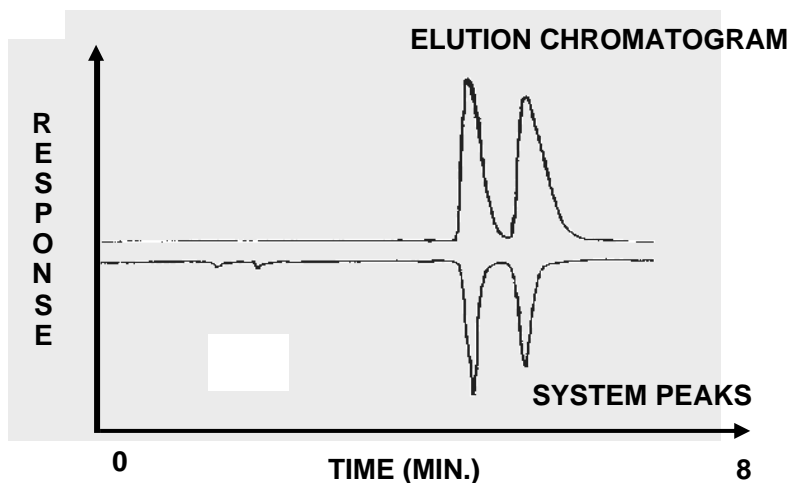
PEAK AREA UNDER NON-COMPETITIVE CONDITIONS
MOBILE PHASE 1:1:1, 0.5mM



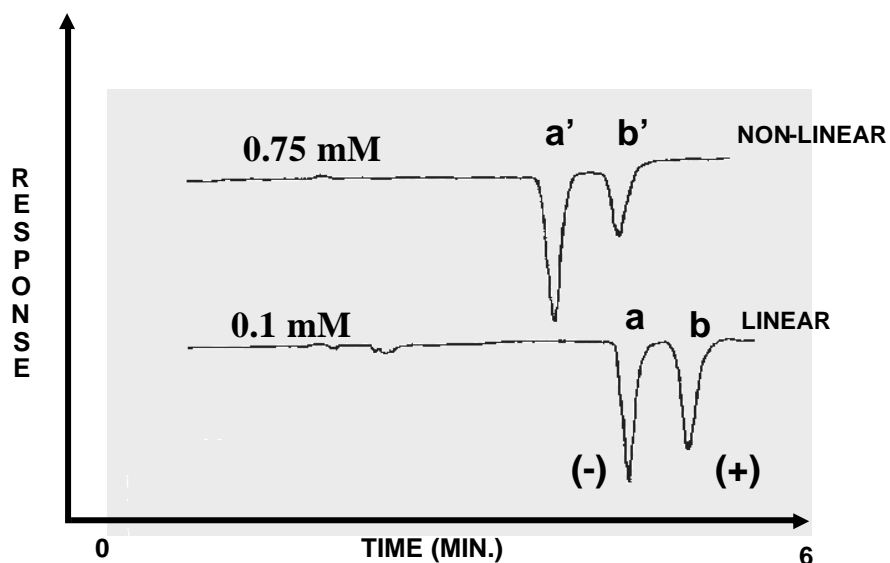
**SYSTEM PEAKS (VACANCY) AT THE
NON-LINEAR RANGE OF
CHROMATOGRAPHY**

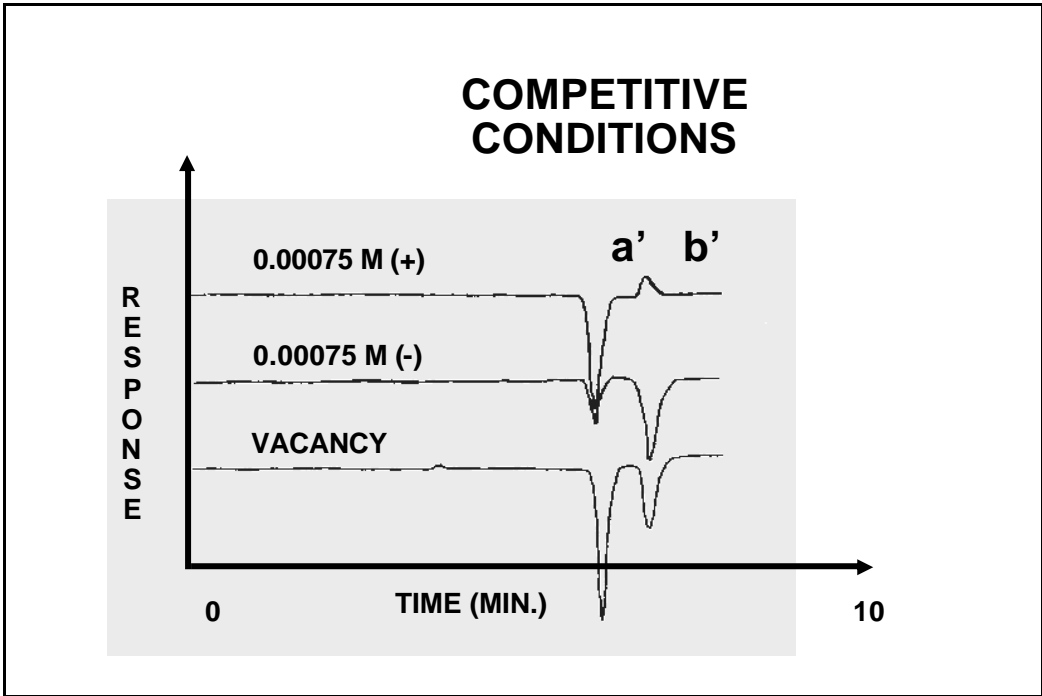
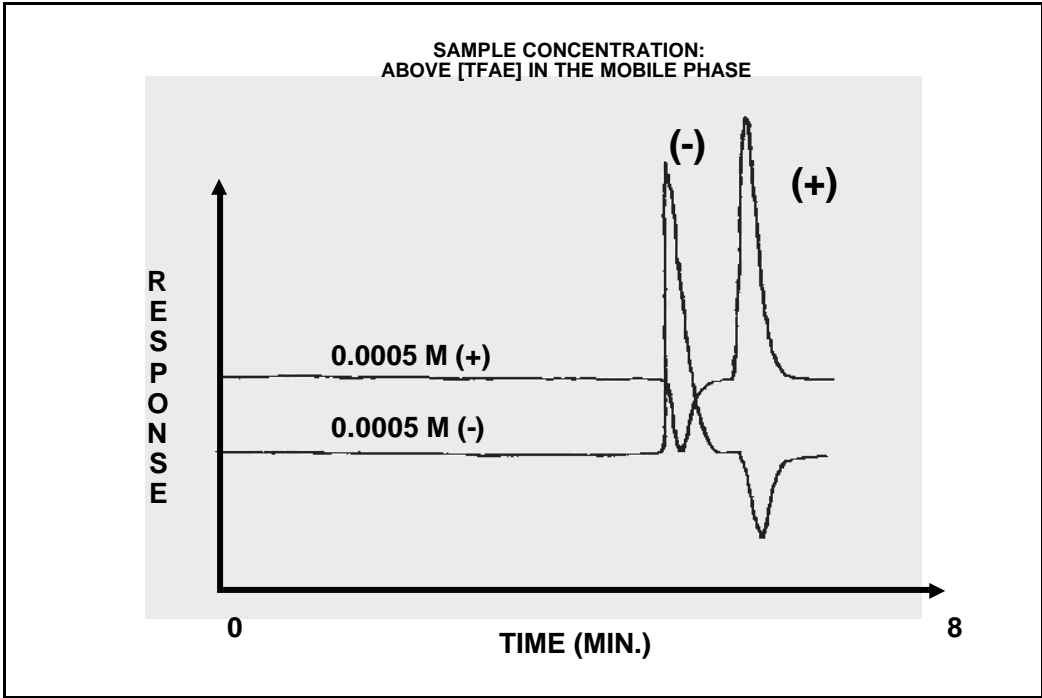


TFAE ENANTIOMERS IN A CHIRAL SYSTEM: SYSTEM PEAKS VS ELUTION

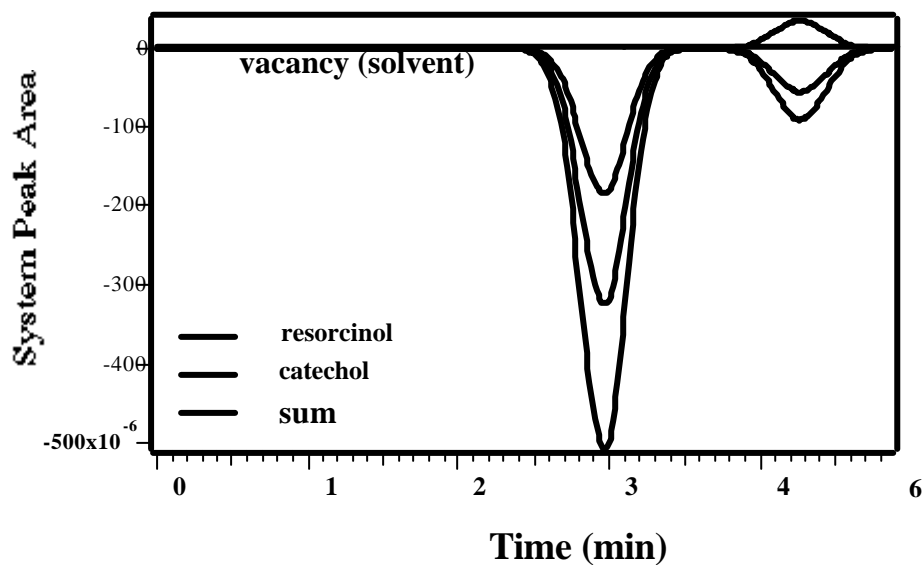


TFAE SYSTEM PEAKS AT LINEAR AND NON-LINEAR CONDITIONS

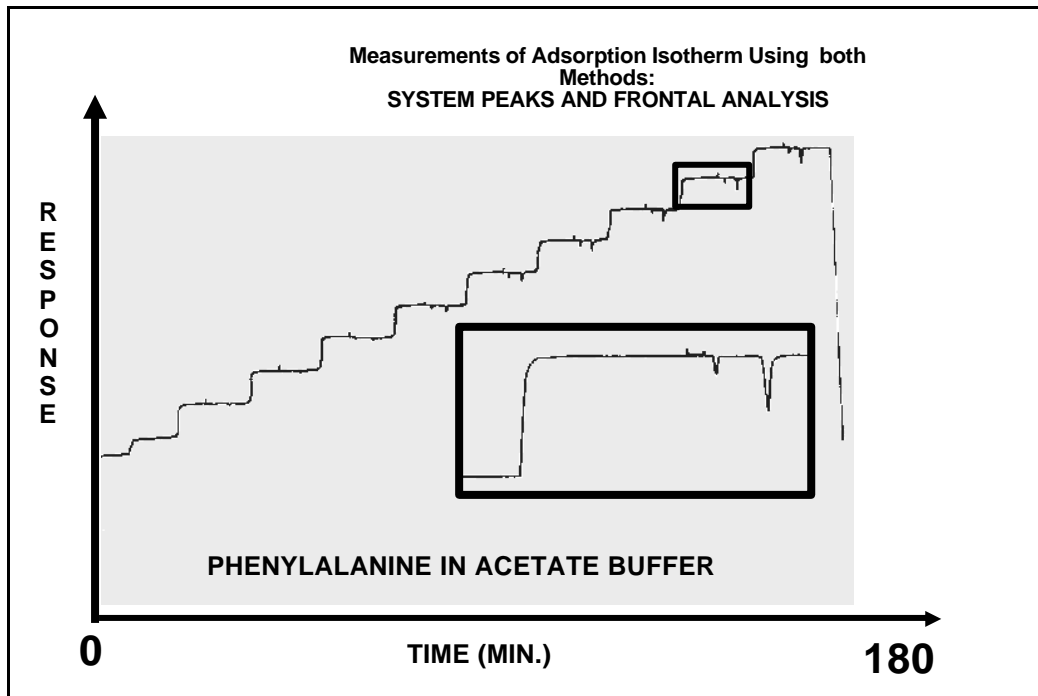




Theoretical Calculations of System Peaks



Practical Uses



Calculation of $C_{S,i}$:

System Peaks Analysis (SPA)

$$C_{S,i} = \frac{1}{\phi} \sum_0^{C_{m,i}} k'_i \Delta C_{m,i}$$

$C_{S,i}$ = concentration in the stationary phase
 k'_i = capacity factor
 f = phase ratio
 $dC_{m,i}$ = difference in concentration between every
two steps.

Frontal Analysis (FA)

$$C_{s,i} = \frac{(V_{R,i} - V_0 - V_h)}{V_s} C_{m,i}$$



STEPWISE:

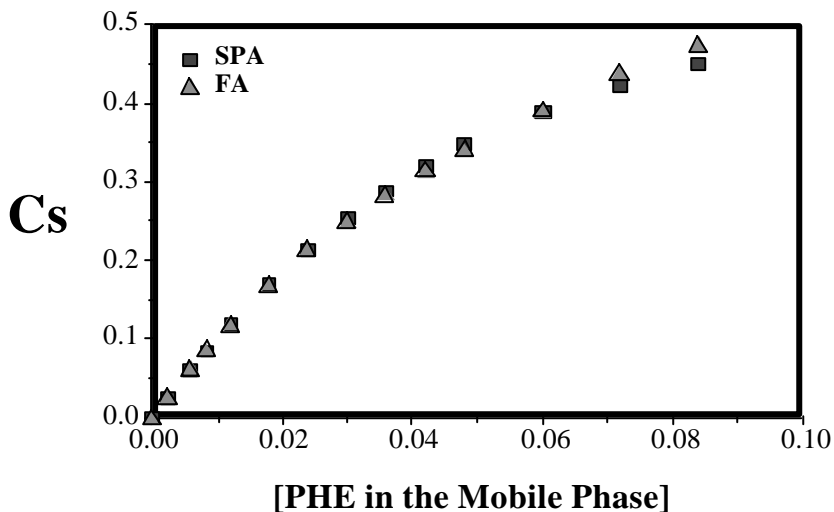
$$C_{s,i} = \sum_0^{C_{m,i}} \frac{(V_{R,i} - V_0 - V_h)}{V_s} dC_{m,i}$$

V_0 = column void volume

V_s = stationary phase volume

V_h = hold-up volume (from the pump to the detector)

ADSORPTION ISOTHERM OF PHENYLALANINE IN 0.1 M ACETATE BUFFER BY FA and SPA



**DETECTION OF IRREVERSIBLE ADSORPTION VIA THE SYSTEM PEAKS
OF THE RESIDUAL ENANTIOMERS**

