System Peaks in Liquid Chromatography

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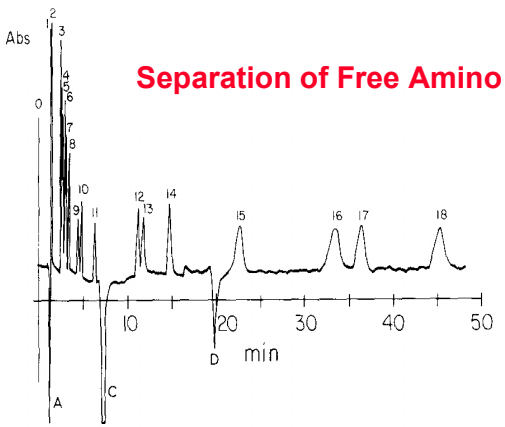
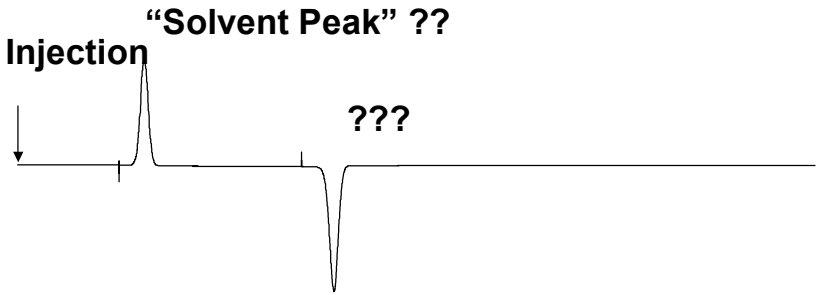


Separation of Free Amino Acids by RP HPLC

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

INJECTION OF PURE SOLVENT



Separation of Free Amino Acids by RP HPLC

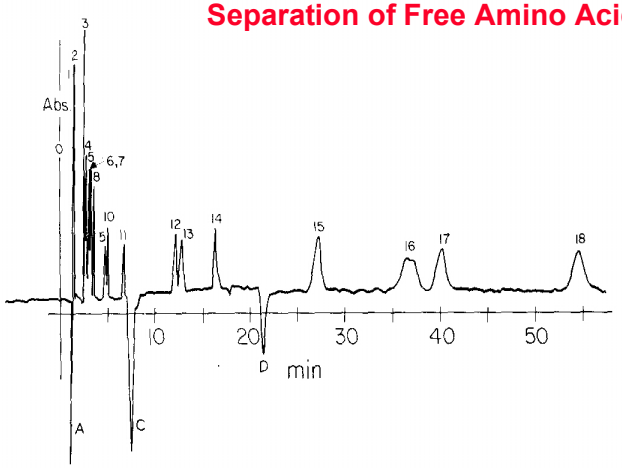


Fig. 7. Chromatogram obtained at 40°C. Mobile phase, 10 m*M* acetate buffer–0.4 m*M* copper(II acetate–0.8 m*M* heptanesulfonate. Other conditions as in Fig. 1. Peaks: 1 = Asp; 2 = Glu; 3 = Gly + Ser; 4 = Asn; 5 = Gln; 6 = Thr; 7 = Ala; 8 = Thr; 9 = αAbu; 10 = His; 11 = Pro; 12 = Val 13 = Nvl; 14 = Met;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

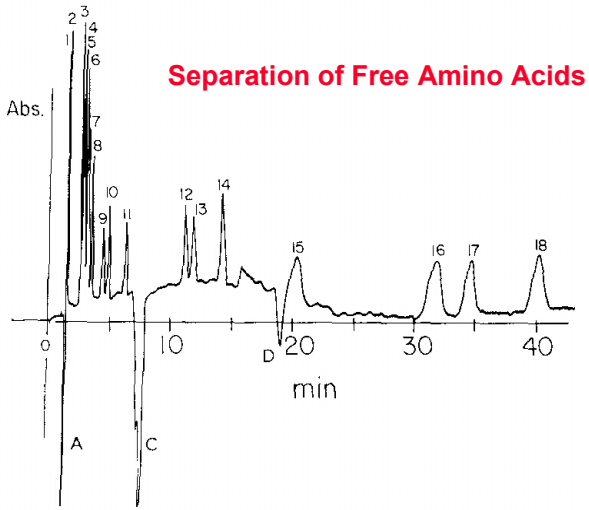
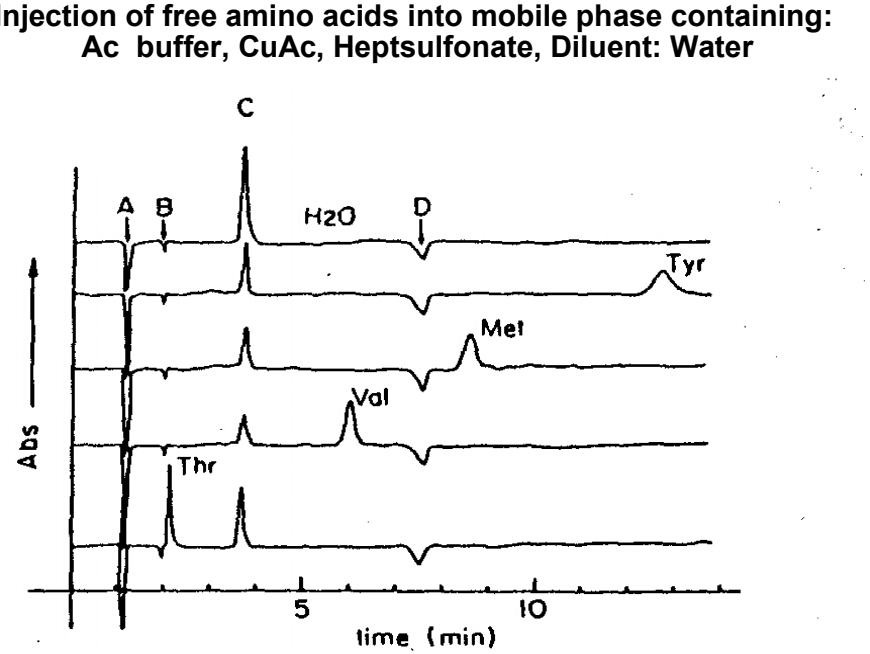
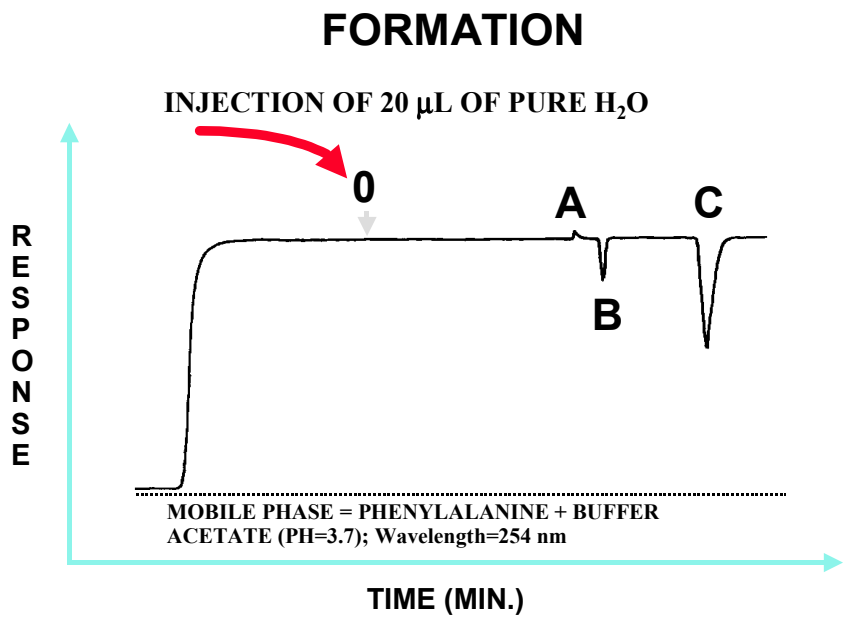
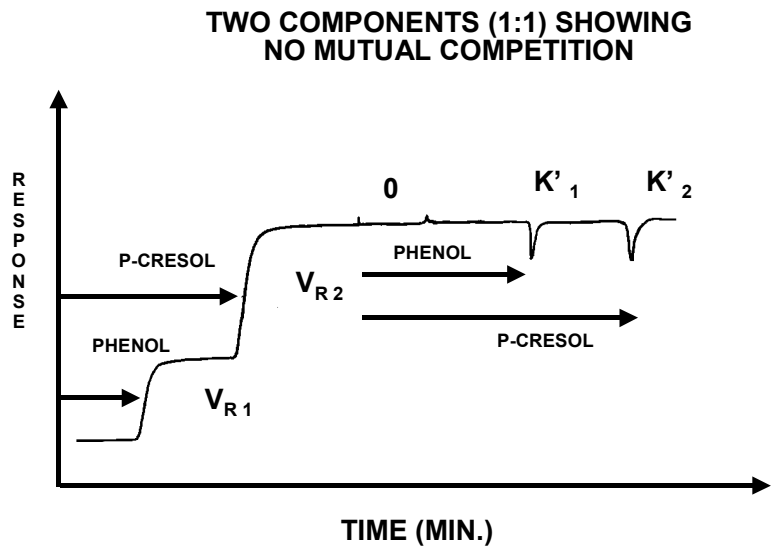


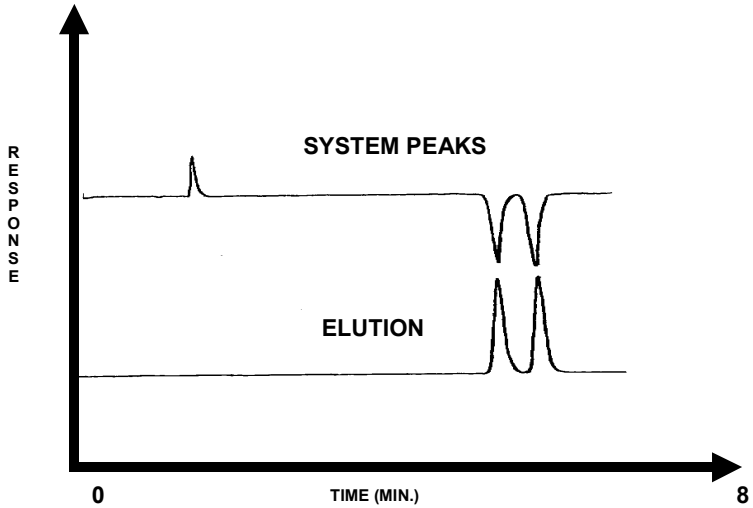
Fig. 9. Chromatogram obtained at 48°C. Conditions and peaks as in Fig. 7.



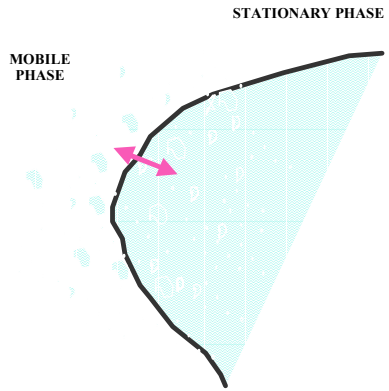




MIXTURE OF (1:1) 1,8- AND 1,5-DCAQ IN THE LINEAR RANGE

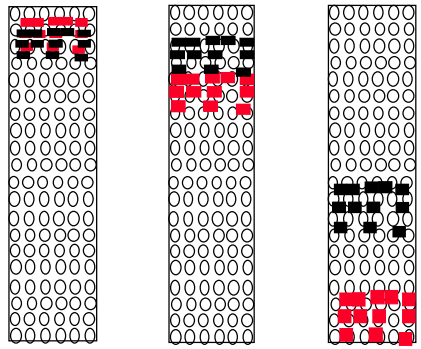


PRINCIPLE OF SEPARATION



$$k' = \frac{t_R - t_0}{t_0} \qquad k' = \phi$$

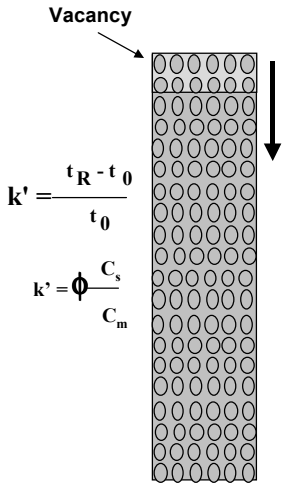
SEPARATION PROCESS:



CONDITIONS FOR APPEARANCE OF SYSTEM PEAKS

- Mobile phase is multicomponent (n=>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.

Mechanism of System Peaks Formation



$$k' = \frac{t_R - t_0}{t_0}$$

$$k' = \phi \frac{C_s}{C_m}$$

EXAMPLE:
Two additives in the mobile phase

Example: k'(1) = 1
Step 1:
Equilibrium:
Cs=1; Cm = 1

Step 2:
Injection of Vacancy:
Cs=1; Cm=0

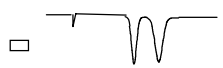
Step 3:
Re-equilibration:
Cs=0.5; Cm=0.5

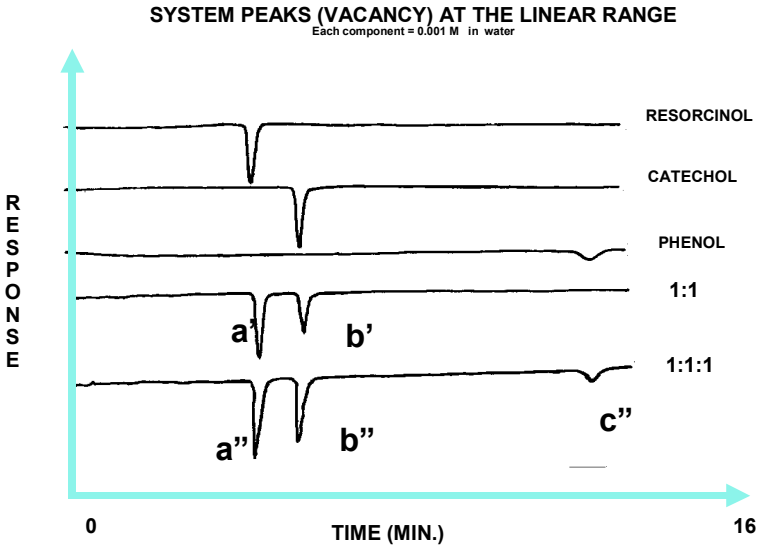
Example: k'(2) = 2
Step 1:
Equilibrium:
Cs=2; Cm = 1

Step 2:
Injection of Vacancy::
Cs=2; Cm=0

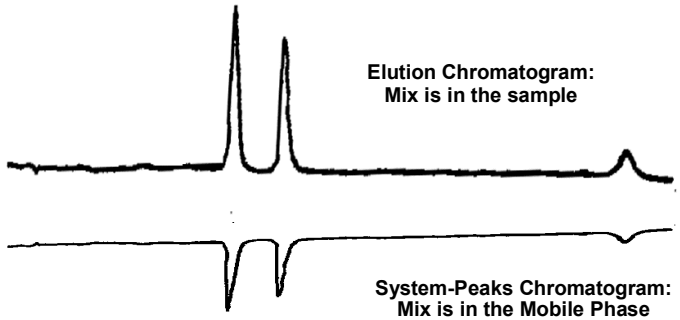
Step 3:
Re-equilibration:
Cs=1.33; Cm=0.67

CHROMATOGRAM

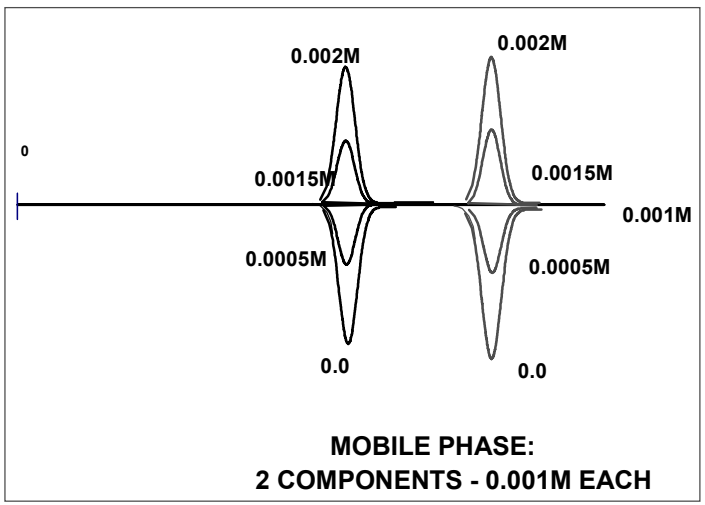




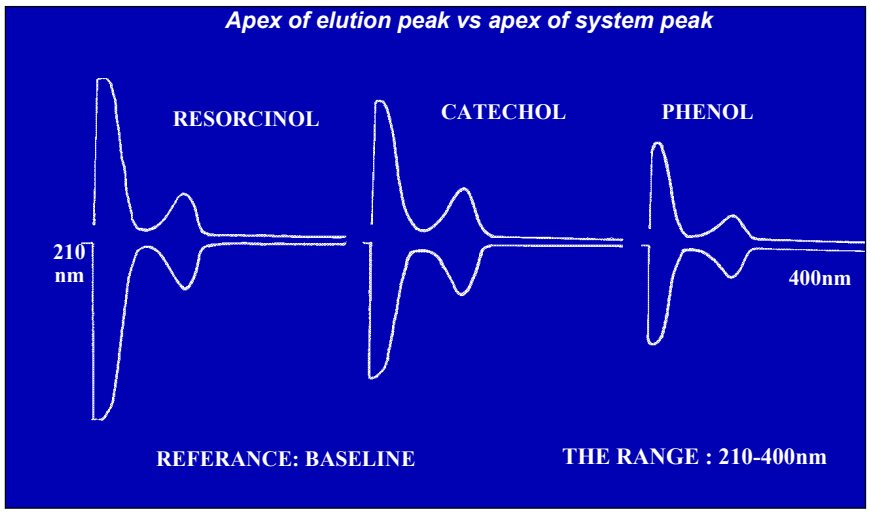
Resorcinol-Catechol-Phenol 1:1:1 Mixture in the Linear Range



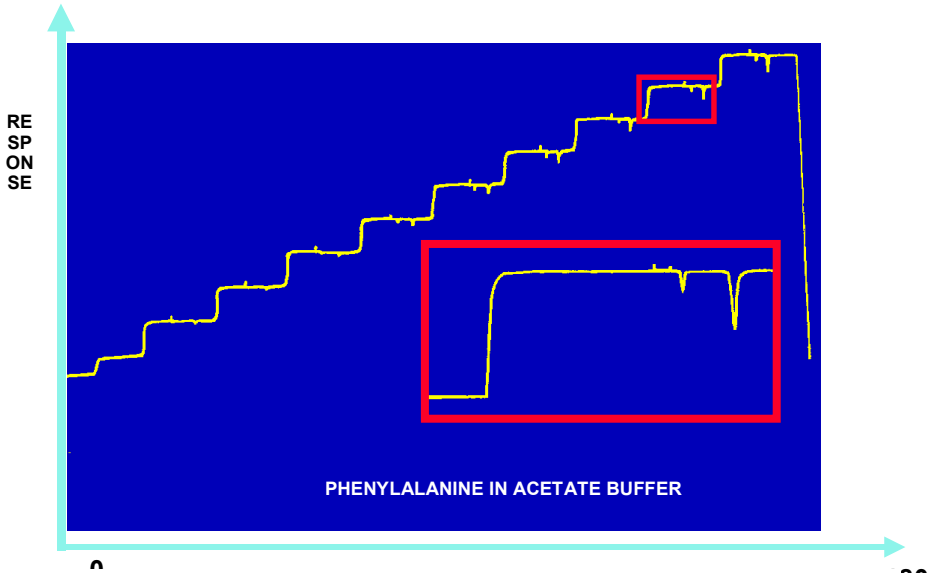
Detection of System Peaks



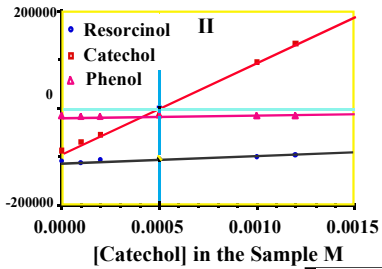
UV-SPECTRUM OF (1:1:1) MIXTURE

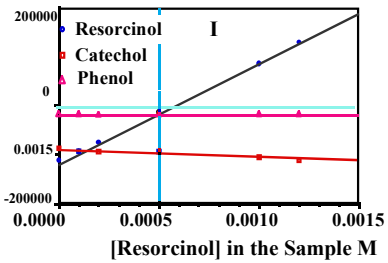


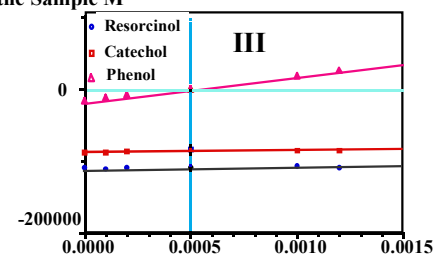
FULL SEQUENCE OF SYSTEM PEAKS AND FRONTAL ANALYSIS



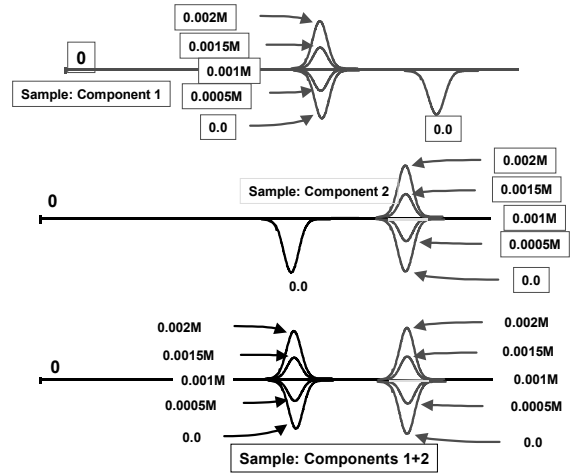
PEAK AREA UNDER NON-COMPETITIVE CONDITIONS
MOBILE PHASE 1:1:1, 0.5mM





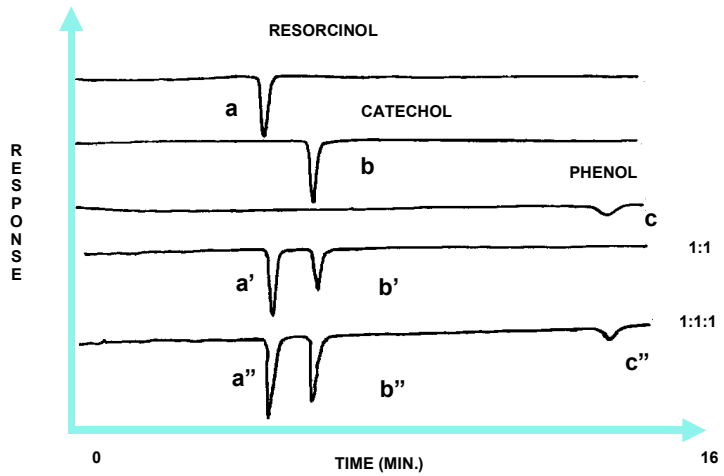


INJECTING MOBILE PHASE COMPONENTS : VARIOUS CONCENTRATIONS IN THE SAMPLE



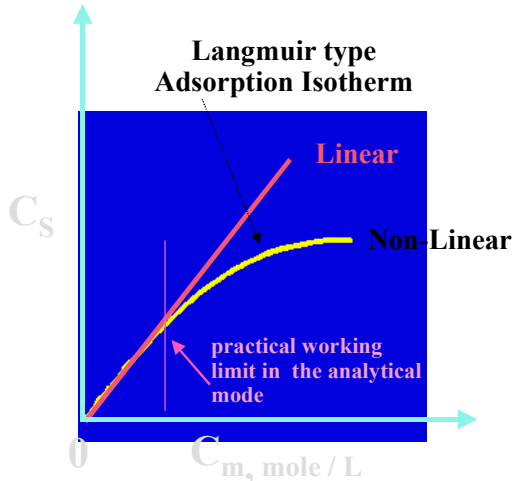
MOBILE PHASE: 2 COMPONENTS 0.001M EACH

MOBILE PHASE'S SOLVENT = WATER



SYSTEM PEAKS (VACANCY) AT THE LINEAR RANGE

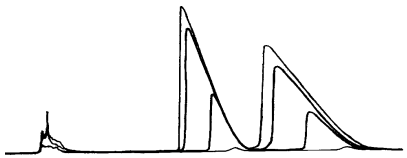
SYSTEM PEAKS (VACANCY) AT THE NON-LINEAR RANGE OF CHROMATOGRAPHY

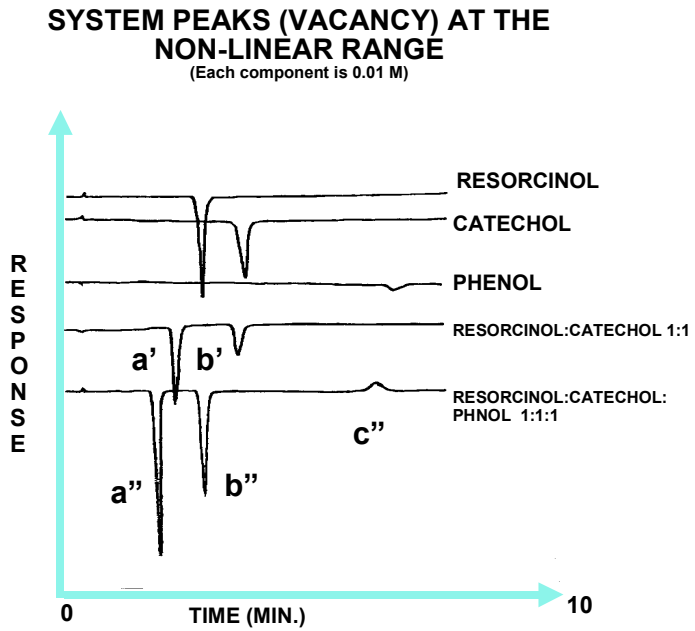


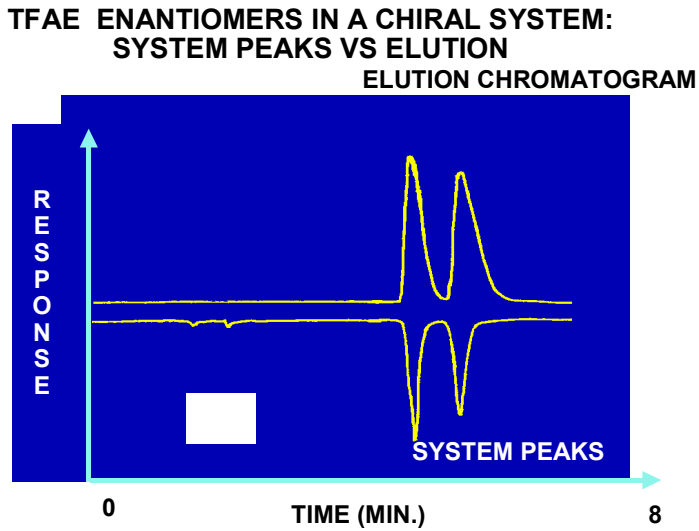
$$C_s = \frac{k'}{\phi} C_m \quad \text{linear}$$

$$k'\left[\phi \frac{C_s}{C_m}\right]$$

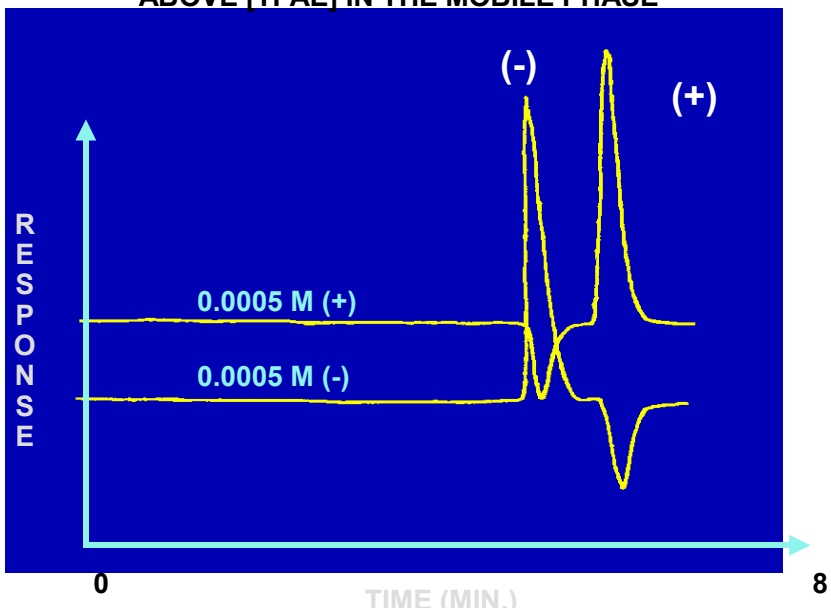
$$C_s = \frac{a\ C_m}{1 + b\ C_m} \quad \text{Non-Linear}$$



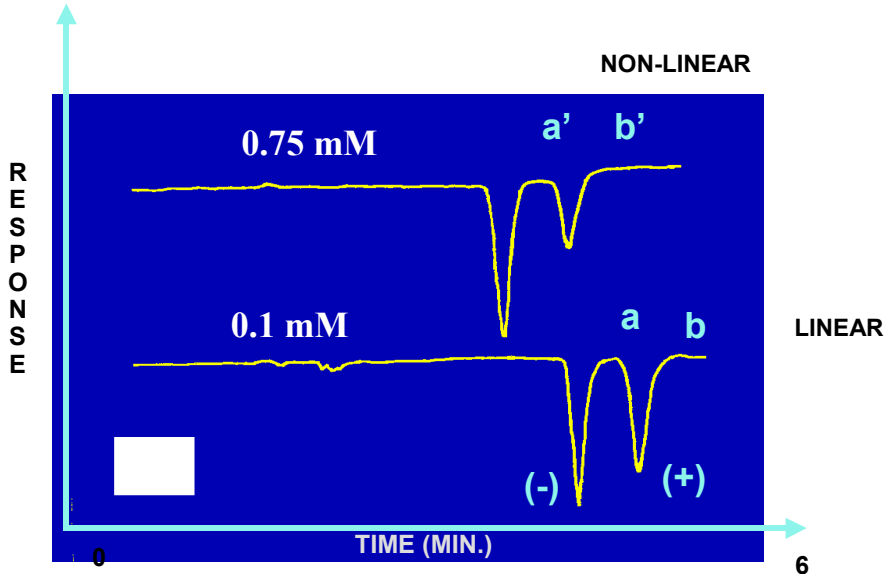




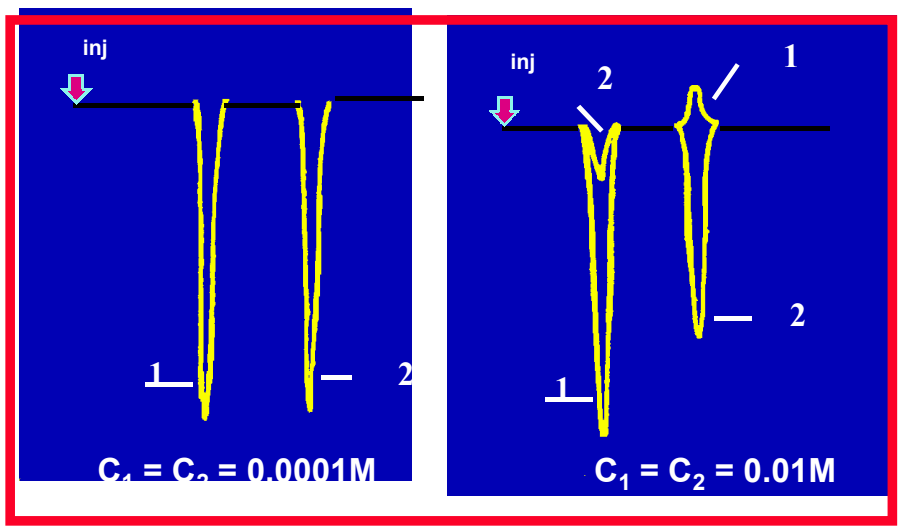
SAMPLE CONCENTRATION: ABOVE [TFAE] IN THE MOBILE PHASE



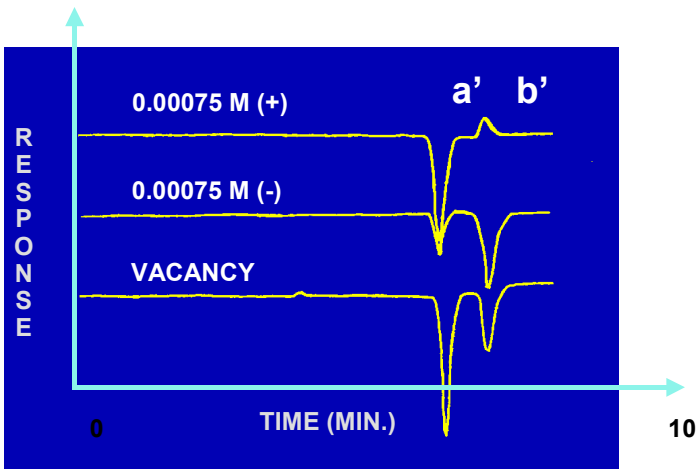
TFAE SYSTEM PEAKS AT LINEAR AND NON-LINEAR CONDITIONS



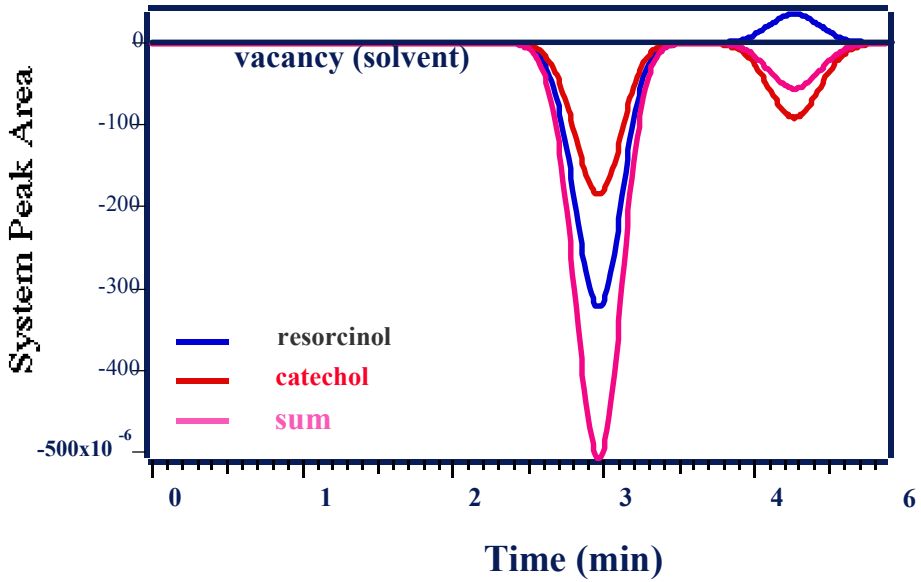
VACANCY CHROMATOGRAPHY IN THE CASE OF (1:1) MIXTURE



COMPETITIVE CONDITIONS



Theoretical Calculations of System Peaks



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