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Use of system peaks for the determination of the distribution of resorcinol, catechol and phenol in liquid chromatography

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Abstract

The single-component adsorption isotherms of resorcinol, catechol and phenol between aqueous solutions and LiChrosorb RP-18 were determined using the system peaks of these components. The single-component isotherms obtained agree well with those derived by frontal analysis. For multi-component solutions at low concentrations, the system peaks obtained are the combination of those observed for the different single-component systems (linear range). When the concentration increases, the retention times and the areas of the different system peaks depend on the nature and concentration of all the system components (non-linear range). The experimental results agree well with the results predicted using the Langmuir competitive isotherms derived from the single-component isotherms. The dependence of the system peak areas on these concentrations is especially complex. The experimental results agree well, however, with those of calculations based on the use of the equilibrium-dispersive model.

1. Introduction

In a number of cases involving most modes of liquid chromatography, the mobile phase contains additives that are retained on the stationary phase [1]. In such cases, an equilibrium is reached between the additive composition of the two phases, and this equilibrium can be perturbed by changes in the column temperature or in the composition of the mobile phase. These perturbations can be used to study the thermodynamics and kinetics of the phase equilibrium. A typical perturbation is the injection of a sample which does not have exactly the composition of the mobile phase. Upon such an injection, two sets of bands migrate along the column and more peaks are recorded than there are sample components. The first set corresponds to the elution of these sample components. The second set corresponds to the perturbation of the additive equilibria. These peaks are called system peaks.

The appearance of system peaks was reported over 20 years ago [2,3]. Systematic investigations of system peaks have been performed by several groups, from different viewpoints [4–11]. The

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origin and characteristics of system peaks, and the variety of information which can be derived from their study, were discussed by Levin and Grushka [12–14]. Theoretical discussions regarding the fundamentals of system peaks were given by Helfferich and Klein [15], Golshan-Shirazi and Guiochon [16] and Riedo and Kováts [17]. Although there is an abundance of papers regarding the observation and the practical uses of system peaks [1], there are few systematic experimental investigations related to the theoretical aspects of the problem.

System peaks are relaxation signals whose study permits an investigation of the underlying equilibria of the additives between the two phases [12]. According to the system peak theory [1,15–17], when the mobile phase contains N components, a weak solvent considered as not adsorbed [18] and $N-1$ retained additives, we observe $N-1$ additive system peaks on injection of a sample pulse or of a vacancy. These peaks propagate at a velocity characteristic of the additives. Note that some of these peaks may not be detectable for a number of reasons [1]. If a P-component sample is injected, we obtain also for each component a peak that moves at a velocity characteristic of the analyte. In this work, we used mainly vacancy chromatography, a technique in which samples of the pure weak solvent are injected. In some experiments, solutions containing mobile phase components of composition different from that of the mobile phase were also injected. Therefore $P=0$, and we have only $N-1$ system peaks. For the sake of simplicity, they are referred to as the system peaks.

An important feature of system peaks is that when the sample size and hence the system perturbation are small, their retention time is independent of the nature of the sample injected. However, the size of the perturbation caused by a given amount of sample depends on the nature of the injected sample. The system peaks can even be positive or negative, depending on the nature of the sample and on the detection mode. Each of these $N-1$ peaks can be assigned to one and only one component of the system only if there is no competition between them for retention. Conversely, when there is competition for retention between the components of the system, the migration of a particular system peak can no longer be related to a specific component. Further, the system peaks are not pure, and each peak contains all of the components involved in the competition, in variable amounts. The migration of each system peak is related to the combination of the influence of all the components. Accordingly, it is difficult to deconvolute or predict the chromatograms.

The object of our work is the investigation of the relationships between the retention times and areas of system peaks and the equilibrium isotherms of all the compounds involved. We used simple, non-ionic systems, with one to three additives, under linear and non-linear conditions. We measured the isotherms by the classical frontal analysis method, and from recorded system peaks under a variety of experimental conditions [19]. The experimental results are compared with those of the theory of system peaks reported previously [1,16].

2. Theory

Assuming the equilibrium-dispersive model which lumps the band broadening effects of finite axial dispersion and a finite kinetics of mass transfer in the column into an apparent dispersion coefficient, the mass balance is written

$$u \cdot \frac{\partial C_j}{\partial z} + \frac{\partial C_j}{\partial t} + F \cdot \frac{\partial q_j}{\partial t} = D_a \cdot \frac{\partial^2 C_j}{\partial t^2} \tag{1}$$

where u is the mobile phase velocity, z is the distance along the column, t is the time, F is the phase ratio [with $F=(1-\varepsilon)/\varepsilon$ and ε is the total column porosity] and D_a is the apparent dispersion coefficient. At equilibrium between the two phases, the concentration of component j in the stationary phase, q_j, is related to the mobile phase concentrations of all the components of the mobile phase by the equilibrium isotherm. Assuming the competitive Langmuir isotherm model for the present case, we have

$$q_j = \frac{a_j C_j}{1 + \sum_i b_i C_i} \tag{2}$$

where a_j and b_j are the numerical coefficients of the pure component Langmuir isotherm of component j. The apparent dispersion coefficient is related to the column HETP by

$$D_a = \frac{Hu}{2} \quad (3)$$

The initial condition is that the concentration of the additive in the mobile phase throughout the column is constant and equal to C_j^0:

$$C_j(z, t = 0) = C_j^0 \quad (4)$$

The boundary conditions correspond to a perturbation of the equilibrium at the column inlet:

$$C_j(z = 0, t) = C_j^P \quad 0 \leq t \leq t_p$$
$$C_j(z = 0, t) = C_j^0 \quad t_p < t \quad (5)$$

where t_p is the duration of the injection pulse and C_j^P is the additive concentration in the injected sample, or perturbation. This latter concentration depends on the experimental conditions. It is zero for a vacancy, but it can be smaller or larger than C_j^0.

According to the theory of system peaks [16], unless the concentrations of all additives are extremely low, the response of the column to a perturbation is complex. It involves the formation of as many peaks as there are additives in the system, even if the perturbation entails a change in the concentration of a single additive. The velocities of the peaks formed are given by the eigenvalues of the matrix of the differentials, $\partial q_i / \partial C_j$, of the competitive isotherm. If the perturbation affects the concentrations of several additives, e.g., of k additives, k series of j system peaks are formed, and the k system peaks formed for each component of the system interfere [16].

3. Experimental

3.1. Instrumentation

Experiments were carried out on an HP1050 (Hewlett-Packard, Palo Alto, CA, USA) liquid chromatograph equipped with a diode-array UV detector and an HPCHEM data station. Injections were done using a Rheodyne (Cotati, CA, USA) injection valve with a 20-μl loop. The temperature was kept constant at 30 ± 0.1°C, by placing the column in the stream of a circulating water-bath. The UV spectra were recorded using a Uvikon 930 instrument (Kontron, Zurich, Switzerland).

3.2. Choice of detection wavelength

Chromatographic detectors are very sensitive. At the concentrations used in this work, the baseline becomes very noisy at the wavelengths of the adsorption maxima in the spectrum which are traditionally used. The precision of the measurements of the retention times and areas of the system peaks is then poor. The wavelength was selected to optimize the signal-to-noise ratio of the system peaks. For all mixtures, detection was carried out at 240 nm for concentrations below 0.01 M and at 298 nm for higher concentrations, as the molecular absorptivities of resorcinol and phenol are negligible and that of catechol is much smaller. In single-component cases, detection was carried out at 240 nm for all concentrations.

3.3. Column

A 125×4 mm I.D. LiChrosorb RP-18 cartridge (Merck, Darmstadt, Germany) was used for all the experiments.

3.4. Chemicals

Water (HPLC grade) was used as the weak solvent in the mobile phase. The additives, phenol, (Merck), catechol and resorcinol (Sigma, St. Louis, MO, USA), were used as received.

3.5. Methods

Experiments were carried out at a flow-rate of 2 ml/min. The chromatographic system was washed with an acetonitrile–water solution between any change in the mobile phase composi-

tion. The data station provided retention times and peak areas.

4. Results and discussion

We studied three binary systems, with water and each of the three additives, resorcinol (a), catechol (b), and phenol (c). One system peak was obtained in each instance (chromatograms 1–3, Fig. 1-I and 1-II). We also studied one ternary system [a 1:1 mixture of resorcinol (a′) and catechol (b′) in water] and one quaternary system [a 1:1:1 mixture of resorcinol (a″), catechol (b″) and phenol (c″) in water]. We obtained two system peaks in the former instance (chromatogram 4, Fig. 1-I and 1-II), and three in the later (chromatogram 5, Fig. 1-I and 1-II). The experiments reported in Fig. 1-I and 1-II were performed under the same experimental conditions, except for the additive concentrations, 0.0005 *M* in Fig. 1-I and 0.01 *M* in Fig. 1-II. The elution order of the three components is resorcinol, catechol and phenol under the analytical conditions.

4.1. Retention of the system peaks

We measured the retention factors of the system peaks $[k' = (t_R - t_0)/t_0]$ and compared them with the theoretical values derived from the isotherms. For binary mobile phases, the retention time of the system peak is easily predicted from the adsorption isotherm of the additive in the weak solvent and its concentration. The area of the system peak is simply equivalent to the sample size, and is proportional to $\Delta n/F_v$, where F_v is the flow-rate and Δn is the amount of additive injected (positive for a pulse, negative for a vacancy). The systematic determination of the system peak retention time as a function of the additive concentration provides a simple method of isotherm determination [19].

When the mobile phase contains several additives, the situation in more complicated, and it is not possible to derive simply the multi-component isotherms from systematic determinations of the retention times of the system peaks. Theory indicates that, under non-linear conditions, the composition of these peaks is mixed, so none of the system peaks in a multi-component mixture can be ascribed strictly to any component of the mixture. The retention times of these system peaks depend on the competitive isotherms of all the additives involved in the competition and on their concentration.

Experimental measurements of the retention factors of system peaks

Measurements of system peak retention times and areas were done for the different binary mobile phases (solutions of phenol, resorcinol or catechol in water), one ternary mobile phase (1:1 resorcinol–catechol mixtures at different total concentrations) and one quaternary mobile phase (1:1:1 phenol–resorcinol–catechol mixtures at different total concentration). The technique of vacancy chromatography was used for the measurements, with injection of small amounts of water.

In the linear range, at concentrations below ca. 0.5 m*M*, two system peaks were recorded for the ternary and three for the quaternary mobile phase (Fig. 1-I). Their retention times were constant, and the same as those of small sample pulses in elution, under linear conditions. As the additive concentrations were increased above 1 m*M*, the retention times of the system peaks began to decrease with increasing additive concentration (Fig. 1-II). This indicates the onset of non-linear behavior, and of competitive interactions between the components of the multi-component mixtures.

Fig. 2-I–III illustrate the dependence of the retention factors of the additives on the concentration of the solutions for resorcinol (Fig. 2-I), catechol (Fig. 2-II) and phenol (Fig. 2-III). The retention factors decrease rapidly with increasing concentration in the range 1–20 m*M*. They continue to decrease, more slowly but steadily, for concentrations up to 100 m*M*. The retention times of the three system peaks were slightly, but significantly, different from those of the pure compounds, demonstrating interactions and hence competition between these components. Thus, the system peaks can no longer be

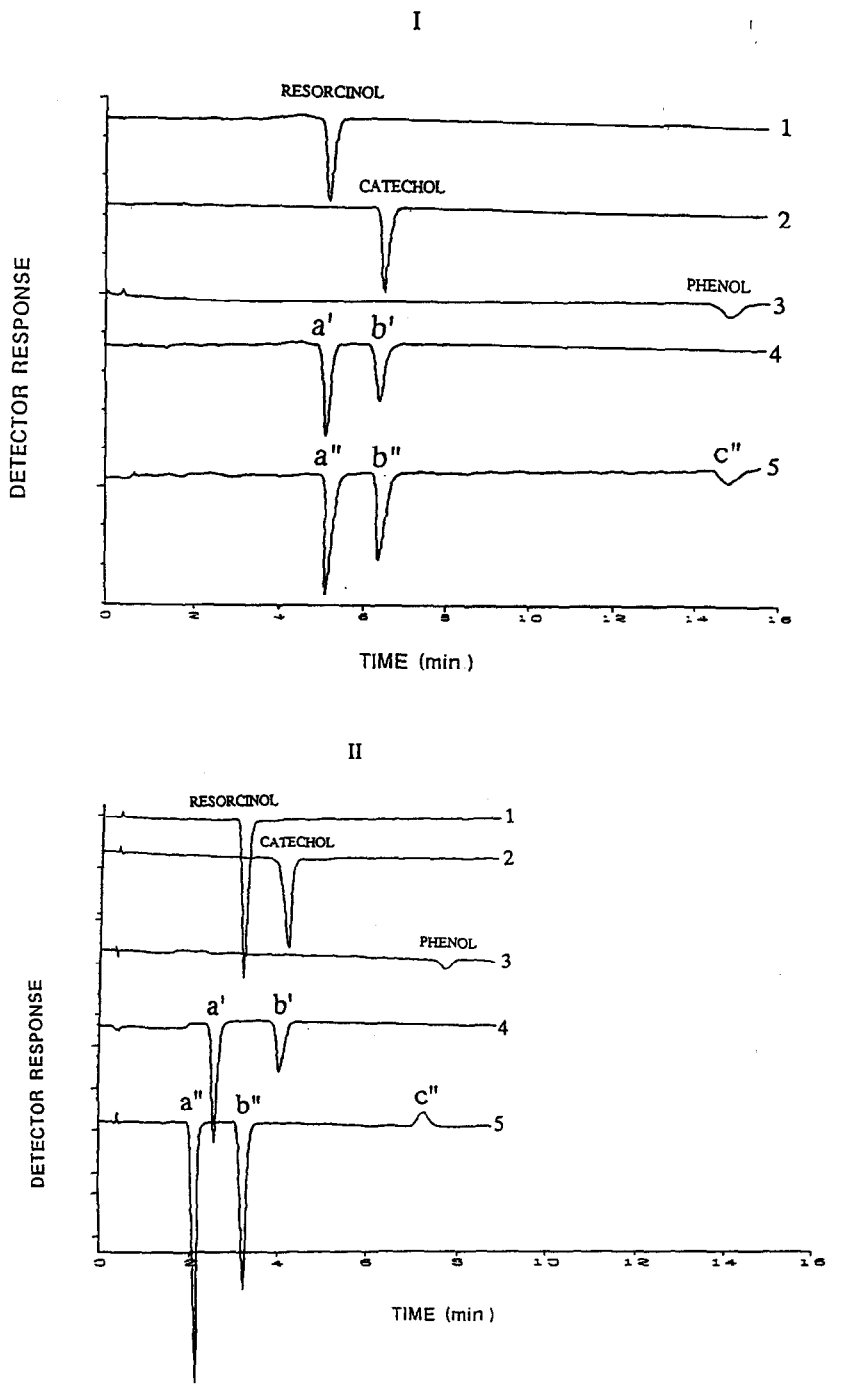


Fig. 1. System peaks obtained on injection of pure water into the following mobile phases: (1) water–resorcinol; (2) water–catechol; (3) water–phenol; (4) water + resorcinol–catechol (1:1); and (5) water + resorcinol–catechol–phenol (1:1:1). Detection at 240 nm in I and II, chromatograms 1–3, and at 298 nm in II, chromatograms 4 and 5. Concentrations: (I) phenol, resorcinol and catechol, 0.5 m*M* each; (II) phenol, resorcinol and catechol, 10 m*M* each.

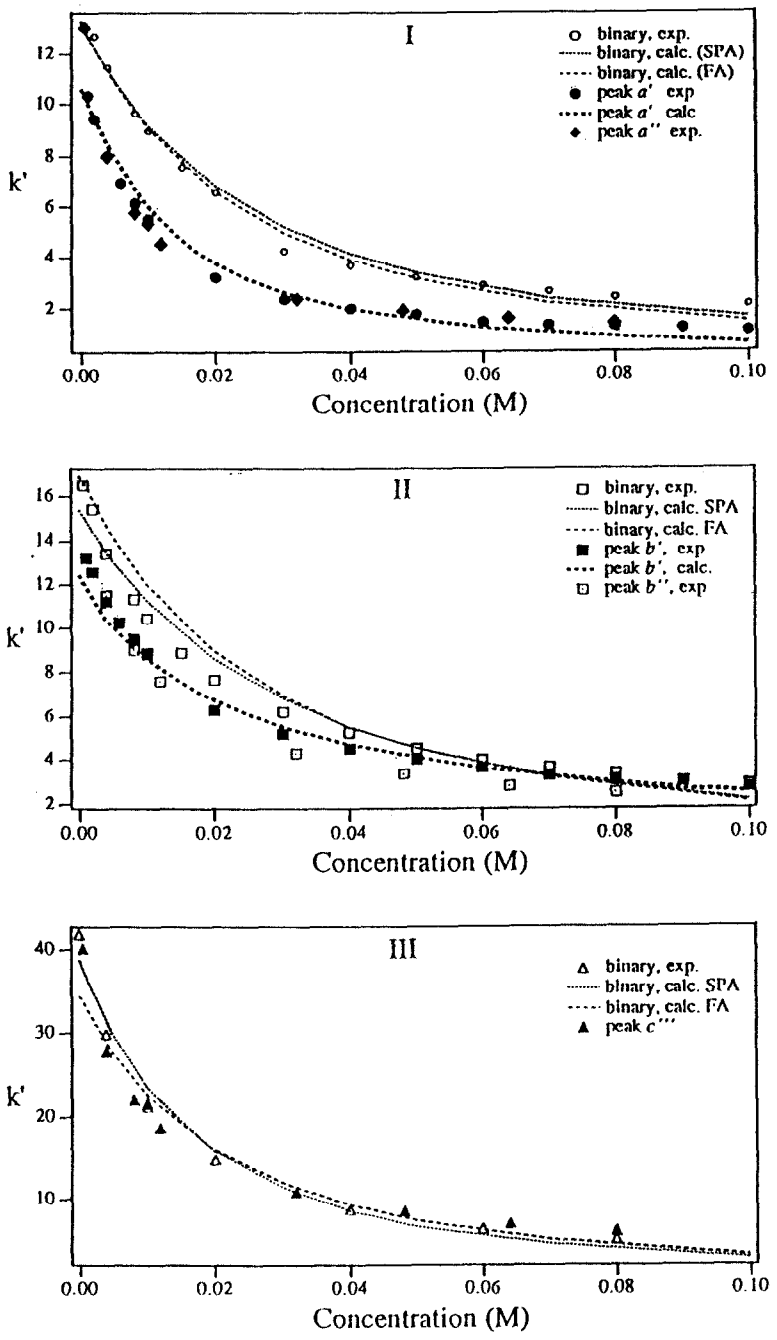


Fig. 2. Retention factors, k', of the system peaks as a function of the concentration of the mobile phase additives. (I) Retention factor of the first peak versus concentration of resorcinol in the binary, ternary (a′) and quaternary (a″) mobile phases; (II) retention factor of the second peak versus concentration of catechol in the binary, ternary (b′) and quaternary (b″) mobile phases; (III) retention factor of the third peak versus concentration of phenol in the binary and quaternary (c″) mobile phases. Experimental results (symbols) and dependences calculated in the binary and ternary systems (lines), using the Langmuir coefficients in Table 1. Sample volume, 20 μl; concentration, 0; column length, 12.5 cm; phase ratio, 1.24; flow-rate, 2 ml/min; t_0, 21 s; column efficiency, 1000 plates.

related to specific compounds. Their migration rates depend on a combination of velocities. Further, the composition of the material transported with the system peaks in ternary and quaternary systems is a mixture of all components, in different concentrations.

In the quaternary mobile phase, for example, each peak contains a certain amount of each of the three additives involved in the competition. This effect is well illustrated in Fig. 1-II, chromatogram 5. As the injection is a vacancy, while all system peaks in linear chromatography are negative (Fig. 1-I, trace 5), the third peak, c″ in this chromatogram, is positive. The signal recorded is the sum of the detector response to the concentration signals of the three additives, resorcinol, catechol and phenol. The balance in peak c″ gives a positive signal. This positive signal is in agreement with the prediction of the system peak theory [16] because phenol and resorcinol do not give a significant detector response at 298 nm. In this instance, the area of peak c″ represents only the excess amount of catechol eluted, and is positive as required by theory.

Determination of adsorption isotherms

The single-component adsorption isotherms were determined by frontal analysis and the results are reported in Fig. 3. This method is accurate, and its results are independent of any assumption regarding the theory of chromatography, provided that the adsorption isotherms are convex upwards, and the band front exhibits a shock layer. The isotherms were also derived by integration of the plots of the system peak retention factor, k', versus the additive concentration, C, obtained for each single-component mixture. The results are also shown in Fig. 3. There is an excellent agreement between the two sets of results.

Table 1
Parameters of the best fit of the adsorption isotherms to the Langmuir equation

Solute	System peaks		Frontal analysis	
	a	*b*	*a*	*b*
Resorcinol	10.6	20.5	10.7	22.2
Catechol	12.4	17.7	13.7	19.8
Phenol	31.2	29.9	27.9	24.7

The isotherm data were fitted to the Langmuir equation. The best coefficients are reported in Table 1 and the corresponding curves are plotted in Fig. 3 (lines). The experimental data (symbols) do not exhibit any systematic deviations from the model at high concentrations. The low

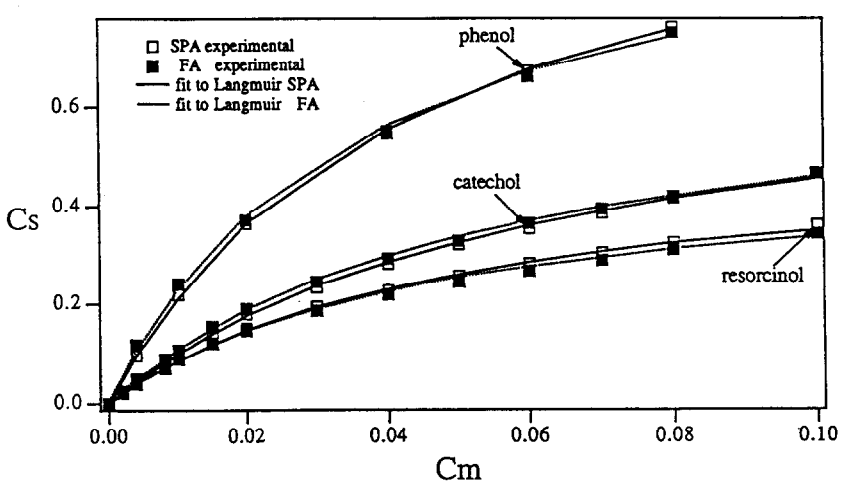


Fig. 3. Adsorption isotherms of resorcinol, catechol and phenol, measured by frontal analysis (FA) and by system peak analysis (SPA). The best values of the parameters of the Langmuir model are given in Table 1.

concentration results (retention times of small pulses in water, initial slopes of the isotherms) are also in satisfactory agreement.

The first parameter, a, of the Langmuir isotherm can also be derived easily from the retention factor of a very small size sample of the additive injected in the pure solvent [$a = k'_0/F = (t_{R,0} - t_0)/(Ft_0)$, where F is the phase ratio]. These values of a are 11.0, 13.3 and 33.5 for resorcinol, catechol and phenol, respectively. Although the experimental isotherm data fitted the Langmuir model well, the values of a derived from the three methods, the fit of the frontal analysis and of the system peak data to the Langmuir model and the measurement of k'_0 under linear conditions, were slightly different for resorcinol. The difference was larger for phenol. This observation is not uncommon and illustrates the sensitivity of the isotherm determination, the problem of fitting experimental data to a two-parameter model [1] and, possibly, the lack of homogeneity of the surface of adsorbents, which are often more active at very low surface coverages.

Validity of the Langmuir competitive isotherm model

The column saturation capacities for resorcinol and catechol are close (Table 1). Thus, the competitive Langmuir model (Eq. 2) is expected to account properly for the competitive adsorption behavior of the two components [16]. Introducing in this equation the coefficients given in Table 1 permits the calculation of the apparent retention factor of a small pulse or vacancy of component j. Using the approach described previously [16], and taking the coefficients of the single component Langmuir isotherms in the calculation, we calculated the retention factors of the three system peaks, a, b, and c, in the binary mixtures and of the system peaks a′ and b′ in the ternary mixtures.

The results of the calculation made for the ternary mobile phase (resorcinol, catechol and water) are shown as the lines in Fig. 2-I and 2-II. These lines are the plots of the retention factors of the two system peaks a′ and b′, versus the concentration of the 1:1 mixture used. As can be seen, there is a substantial agreement between the experimental data and the retention factors derived from the competitive Langmuir isotherm model. This justifies the theoretical approach [16], and the use of the Langmuir competitive model to account for the adsorption behavior of the two components in the present experimental case.

A similar approach could not be extended to the competitive adsorption behavior between phenol and resorcinol and catechol. The saturation capacities of the adsorbent for phenol and the other two components are too different to allow the use of the competitive Langmuir model to account for their competitive interactions.

4.2. System peak areas

Although the retention of system peaks has already been used extensively in their study in connection with isotherm determination, for example, their areas have been far less systematically investigated. Peak areas were measured under non-competitive and competitive conditions for the various system peaks recorded. We assumed that the detector response is linear.

System peak areas under non-competitive conditions

Under linear conditions, each system peak can be ascribed to one mobile phase component, hence the peak area is proportional to the size of the disturbance, i.e., to the extent of the vacancy created by the injection provided that the detector response is linear. The peak area is independent of the nature and concentration of the other components of the sample. The spectra of the three components studied are given in Fig. 4-I and 4-II. The positive spectra in Fig. 4-I were recorded from the top of small elution peaks and the negative spectra were recorded from the trough of the vacancy peaks. Fig. 4-I(1) shows the spectra obtained with binary mixtures, i.e., with pure additives at a concentration of 0.5 mM. Fig. 4-I(2) shows the spectra in the case of the quaternary mixture, with concentrations of 0.1 mM for each additive. There are virtually no

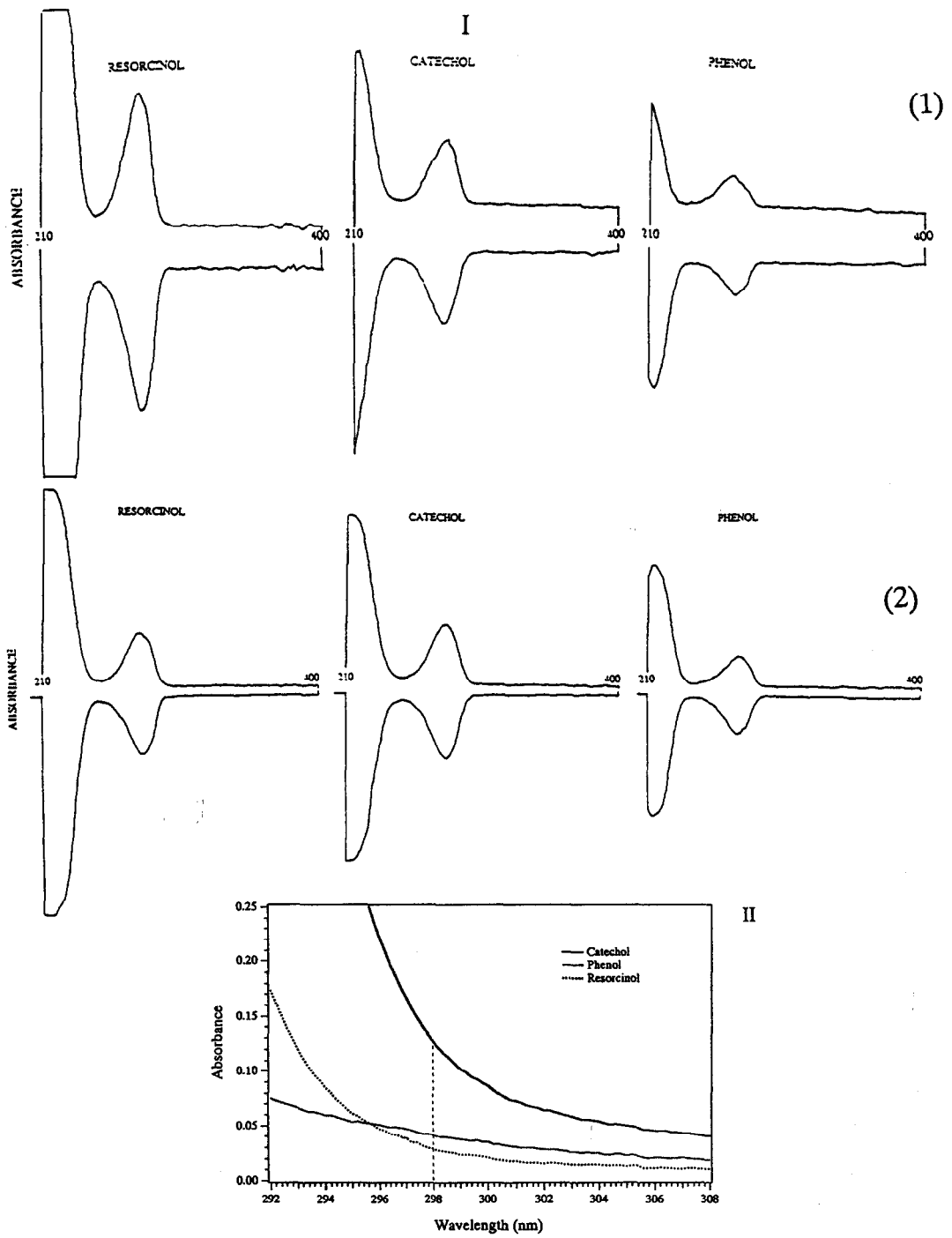


Fig. 4. UV spectra of the compounds studied. (I) Spectra (210–400 nm) recorded with the diode-array detector on the top of the peaks of resorcinol, catechol and phenol, in the case of analytical-size injections (positive peaks) and vacancy injections (negative peaks). 1 = Binary systems; 2 = quaternary system. Reference, baseline. (II) UV spectra of resorcinol, catechol and phenol (10 m*M* each), recorded with a UV spectrophotometer and overlaid, in the region 292–308 nm, showing the low response for resorcinol and phenol at 298 nm.

differences, and the positive (elution in water) and negative (vacancy) spectra are mirror images. These observations confirm our earlier conclusions regarding the lack of competitive interactions between these components at low concentrations (below 1 mM).

Small samples of water containing only one of the three additives in the quaternary mixture were injected, instead of the standard vacancy injected in previous experiments. The concentration of this single additive was increased while the vacancy of the other two components remained constant. The peak area was plotted versus the amount injected. The experiment was repeated for each additive. The results are plotted in Fig. 5-I–III. They are in agreement with similar, previous results obtained with ionic systems [12–14]. As expected, the peak area of only one additive changes during these experiments. It is the area of the additive whose concentration in the sample was changed during the series of experiments. The calibration line intersects the abscissa axis at $C = 0.5$ mM, the additive concentration ion the mobile phase.

System peak areas under competitive conditions

Experimental results with the ternary mobile phase. At high additive concentrations, competitive adsorption behavior results in a more complex situation [16]. The chromatogram obtained depends on the number of components and their elution order compared with the additive system peak itself [1]. If the detector responds only to the additive (indirect detection), the system peaks associated with each component are positive when the components are eluted before the additive system peak and negative when they are eluted later [16,17]. The additive system peak is negative if all the components are eluted before it, and positive if they are all eluted after it. If some components are eluted before the additives and others are eluted later, the sign and size of the additive system peak result from a mass balance of all the perturbations, positive or negative, caused by the injection of the sample. The total algebraic area of these perturbations is

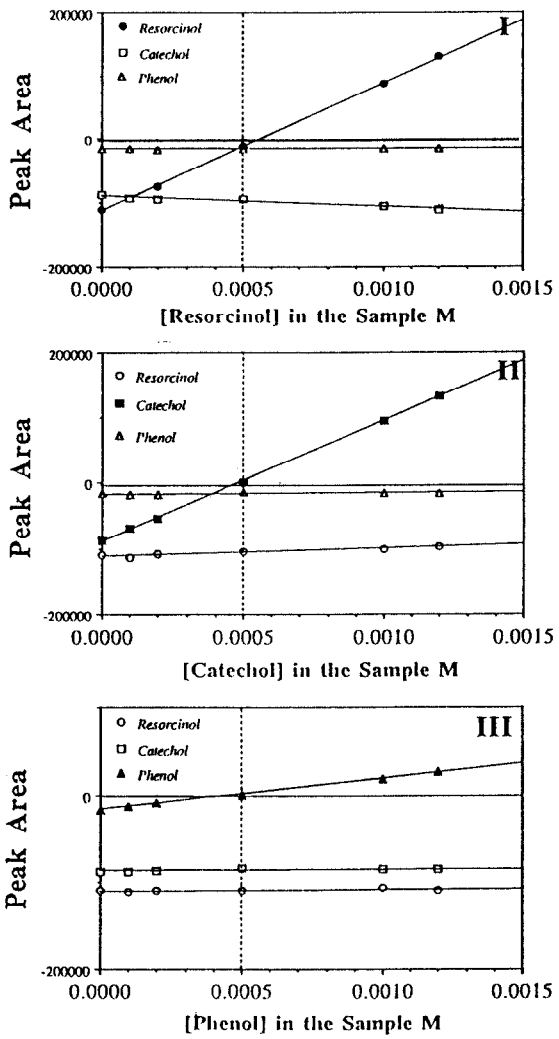


Fig. 5. Plots of the areas of the three system peaks as a function of the additive concentrations. (I) Resorcinol; (II) catechol; (III) phenol. Mobile phase, resorcinol–catechol–phenol (0.5 mM each) (1:1:1). Detection at 240 nm.

equivalent to the detector response for the amount Δn of additive.

As seen in Fig. 6, the areas of all the system peaks vary with increasing concentration of any one of the additives in the sample volume. This is simply explained by the fact that at high concentration each system peak contains every component of the system, albeit in varying amounts. The results shown in Fig. 6 were obtained with the ternary mobile phase (1:1

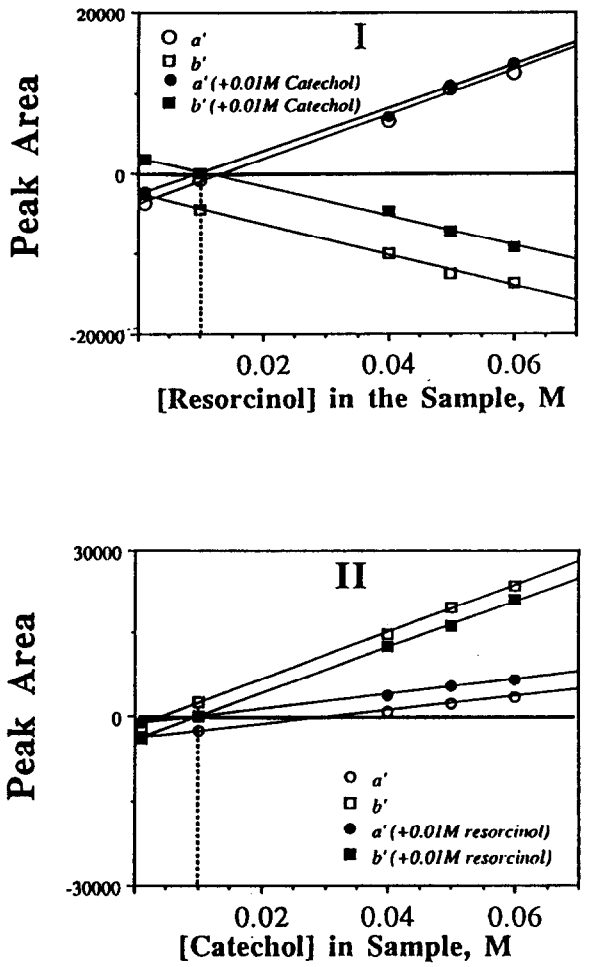


Fig. 6. Plots of the areas of the two system peaks, a′ (circles) and b′ (squares), as a function of the additive concentrations. (I) Resorcinol at various concentrations (open symbols) and same resorcinol solutions with 10 m*M* catechol (solid symbols). (II) Catechol at various concentrations (open symbols) and same catechol solutions with 10 m*M* resorcinol (solid symbols). Mobile phase, resorcinol–catechol (10 m*M* each) (1:1). Detection at 298 nm.

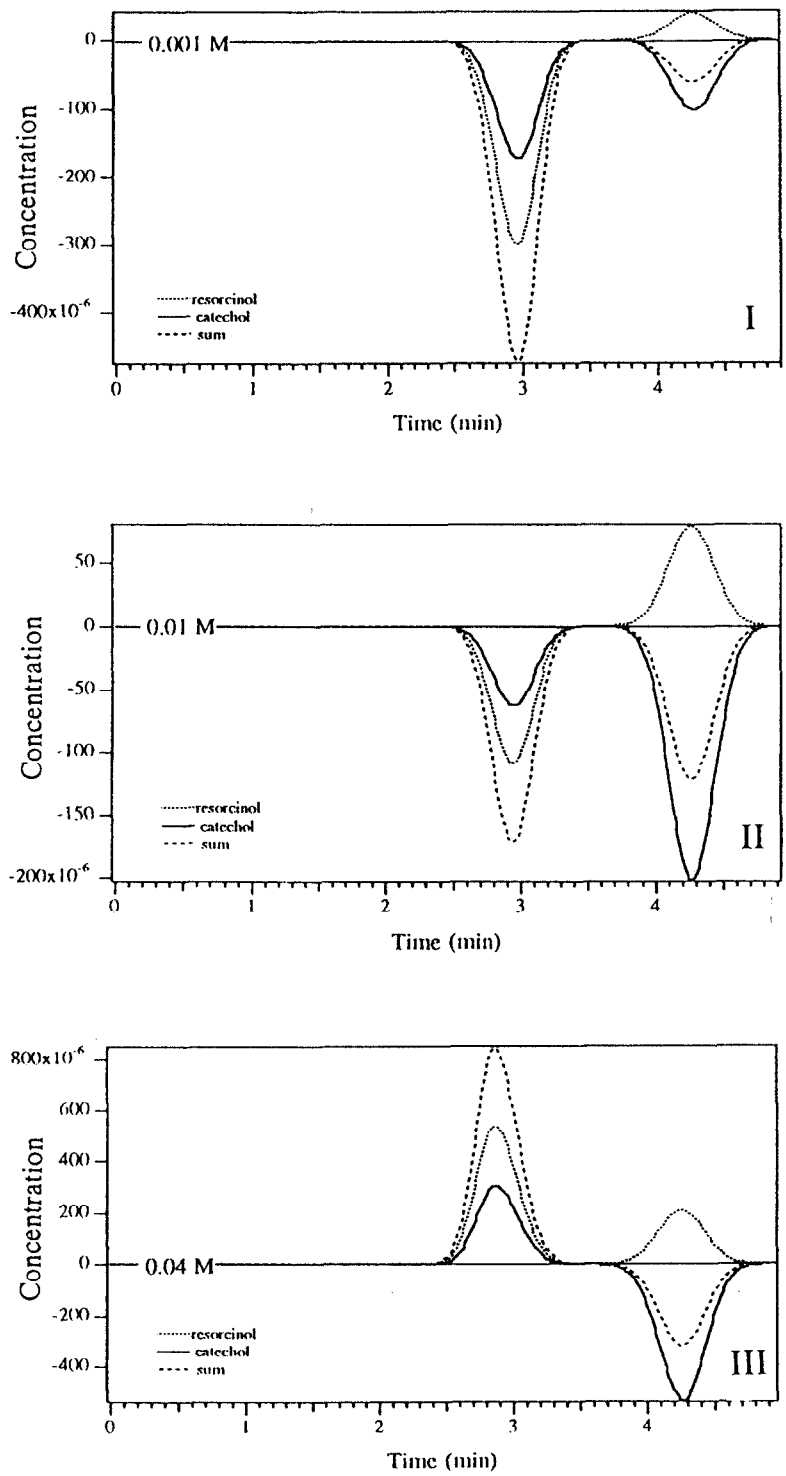
resorcinol–catechol at 10 m*M* each). When solutions of increasing concentrations in resorcinol were injected, the area of the first system peak (a′) increased linearly, whereas the area of the second system peak (b′) decreased linearly. Adding a constant concentration, 10 m*M*, of catechol to the sample shifted the response curve of the second system peak by a fixed, positive amount, whereas it hardly changed the response for the first system peak. Conversely, when solutions of catechol of increasing concentrations were injected, the areas of both system peaks increased linearly. Adding a 10 m*M* resorcinol concentration to the sample shifted the first peak response upwards and the second peak response downwards by fixed amounts.

If a sample of one of the two additives having a concentration equal to the additive concentration in the mobile phase is injected, the corresponding system peak does not vanish. Its area does not become zero (Fig. 6), in contrast to what happens under linear conditions (Fig. 5). Only when the sample and the mobile phase have the same composition does the system peaks vanish. This effect is due to the presence of both additives in each of the two system peaks.

The influence of competitive adsorption behavior on the area of system peaks becomes noticeable at concentrations above 0.5 m*M*. We also measured UV spectra as in Fig. 4-I(2) in a quaternary system at 0.5 m*M* each. The spectra were no longer exact mirror images of each other. This demonstrates the occurrence of some non-linear effects at a concentration of 0.5 m*M* in each of the three additives.

Calculated chromatograms for the ternary mixture. The chromatograms from which Fig. 6 was derived were calculated using the algorithms described previously [16] and the competitive Langmuir isotherm derived above, for a 10 m*M* mobile phase concentration. The chromatograms obtained are given in Fig. 7-I–VII. The concentrations of the two components (resorcinol and catechol, respectively) in the samples were lower than in the mobile phase in the chromatograms in Fig. 7-I and 7-VI, and higher in the chromatograms in Fig. 7-III and 7-VI. In the chromatograms in Fig. 7-II and 7-V these concentrations were the same. The vacancy chromatogram was also calculated, and is shown in Fig. 7-VII.

Although both phenol and resorcinol absorb at 298 nm (see Fig. 4-II), their response factors are low compared with that of catechol. For the sake


0.001 M
Concentration
0
-100
-200
-300
-400x10^-6
resorcinol
catechol
sum
I
0
1
2
3
4
Time (min)
0.01 M
Concentration
50
0
-50
-100
-150
-200x10^-6
resorcinol
catechol
sum
II
0
1
2
3
4
Time (min)
0.04 M
Concentration
800x10^-6
600
400
200
0
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-400
resorcinol
catechol
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III
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Time (min)

Fig. 7.

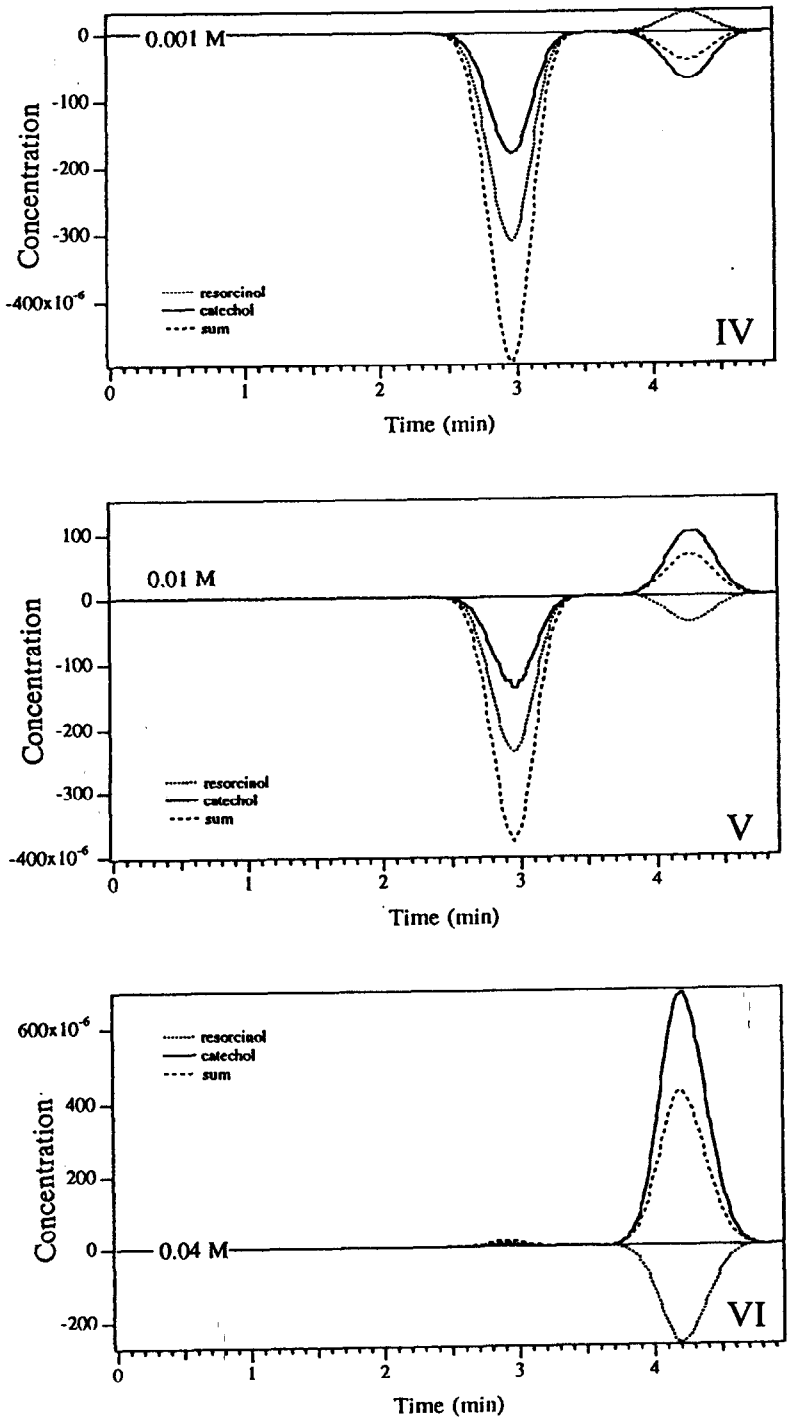

Fig. 7. *(Continued on p. 226)*

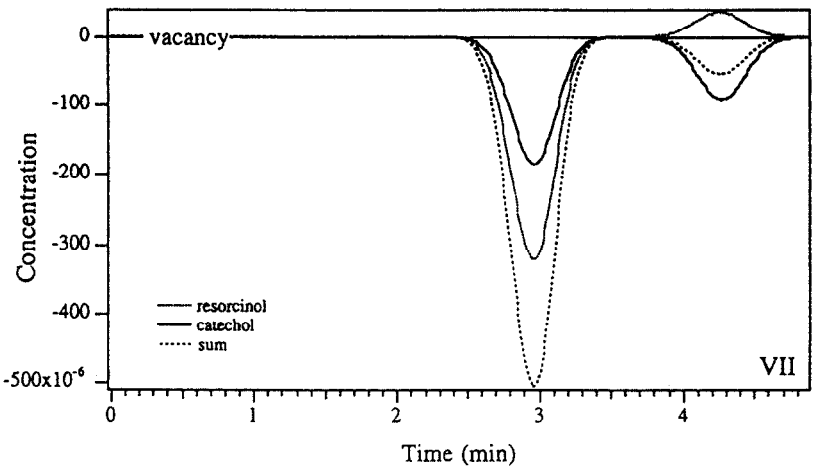

Fig. 7. Calculated chromatograms showing the system peaks predicted by the system peak theory for the ternary mixture, with a mobile phase containing 10 mM each of resorcinol and catechol. Samples: I–III, resorcinol; IV–VI, catechol; VII, pure water. Sample volume, 20 μl; concentrations as indicated. L, F, F_v, t_0, N and sample volumes as in Fig. 2.

of simplicity in the following discussion, we assume that the responses of phenol and resorcinol are negligible compared with that of catechol. This is not entirely true and a small correction should be applied to the quantitative results. The nature of the argument would remain unchanged, however. The comparison of Figs. 1-II (chromatogram 4) and 7-VII shows clearly that the profiles calculated for catechol, the only compound which is detected by the UV detector at 298 nm (Fig. 4-II), are nearly identical with the experimental chromatograms. The same agreement was observed in all other experiments (not reported). The areas of the system peaks were derived and are plotted against the sample concentration of resorcinol and catechol in Fig. 8-I–III and 8-IV–VI, respectively. The plots of the experimental data in Fig. 6-I–II and of the calculated data in Fig. 8-IV–VI are very similar. The experimental results reported in Fig. 6 can now be explained in detail, assuming that catechol is the detection agent for the system peaks recorded at 298 nm, and that the "resorcinol" peak recorded is in fact due to its catechol content. The small contribution of the UV absorbance of resorcinol is neglected.

When resorcinol was injected, the detected signals are the two perturbations to the catechol equilibrium, the one which travels with the catechol vacancy and the one which travels with the resorcinol pulse. When the injected amount of resorcinol increases (Fig. 6-I), the amount of catechol expelled from the stationary phase by the competitive effect of the increasing amount of resorcinol also increases, and the absolute value of the area of the corresponding (negative, b′) peak increases, hence the area of the first system peak (peak a′), increases (Figs. 6-I and 8-I–III). Assuming that catechol is the only detection agent of peaks a′ and b′, the area of peak b′ can be determined by observing that the sum of the areas of the peaks a′ and b′ is equivalent to the injected amount Δn of catechol, i.e., to the difference between the amounts of catechol in the vacancy pulse injected and in an equal volume of mobile phase. Similarly, when the injected amount of catechol increases, the areas of all recorded peaks increase (Fig. 6-II), although the amount of resorcinol in the second system peak actually decreases (Fig. 8-IV–VI), but the resorcinol response is negligible in these experiments.

As seen in Fig. 6, the slopes of the plots of the peak areas versus injected amounts of resorcinol or catechol were the same whether the injected pulse contained or not a constant amounts of the other hydroxyphenol. The addition of the other component merely caused a vertical shift in the position of the straight line obtained. The addition of a constant amount of catechol to the

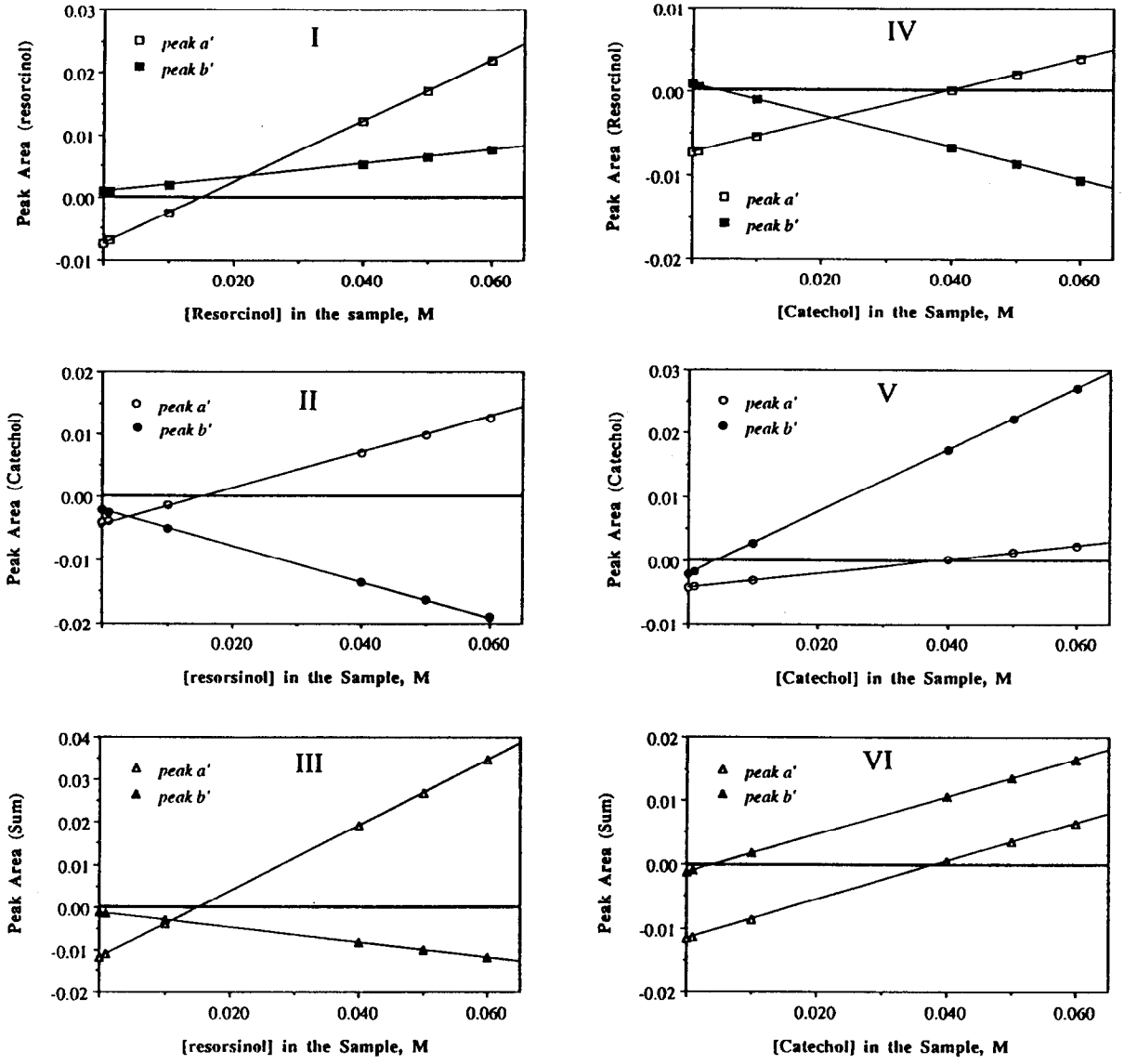

Fig. 8. Plots of the area of the calculated system peaks versus the sample concentration. Samples: I–III, resorcinol; IV–VI, catechol. Mobile phase composition as in Fig. 6.

resorcinol pulses caused a positive shift on the two response curves, confirming the distribution of catechol between the two peaks, and the fact that it is the catechol response which is recorded. The addition of a constant amount of resorcinol to the catechol pulses caused a positive shift of the first and a negative shift of the second peak response factor. This illustrates the influence of resorcinol on the distribution of catechol between the two system peaks, and further confirms the presence of both components in both system peaks.

Study of a quaternary mobile phase. The dependence of the system peak areas on the sample size was also studied for the quaternary mobile phase. Constant volumes of samples of increas-

ing concentrations in resorcinol, catechol or phenol were injected in a mobile phase containing an equal concentration (10 mM) of each of the three phenols. The results are shown in Fig. 9-I–III. The interpretation of the results is greatly simplified by the assumption that in each instance the signal recorded is the variation of the catechol concentration, and that the small contributions of phenol and resorcinol are negligible. Because the concentrations of the components of the mobile phase are high enough for the equilibrium behaviour to be non-linear, a change in the catechol concentration is associated with each of the three system peaks. This change gives rise to the three peaks observed and measured. The same three system peaks also appeared in vacancy chromatography, when a sample of pure water was injected, but the first two were negative and the third one positive (Fig. 1-II, chromatogram 5). This result is in agreement with the system peak theory [16], since the UV detector responds mainly to catechol at this wavelength (Fig. 4-II).

When a sample of resorcinol is injected (Fig. 9-I), the area of the third peak remains nearly constant, whereas the areas of the other two peaks follow the same trend as for the ternary mixture (Fig. 6-I). The area of the first peak increase whereas that of the second peak decreases with increasing sample size, in agreement with the system peak theory [16]. This confirms that the competition between resorcinol and catechol is intense, as demonstrated above. Because the response of resorcinol is negligible, however, no further conclusions can be derived from the fact that the area of the third peak, i.e., the amount of catechol travelling with it, is constant. Especially, we cannot draw conclusions regarding the lack of competition between phenol and the other two additives.

The injection of catechol samples leads to very similar conclusions (Fig. 9-II). The area of the third peak is small and remains nearly constant. The area of the other two peaks increase, the first more slowly than the second, which is to be expected as catechol contributes much more to the second than to the first peak. Finally, the injection of phenol samples results in a marked

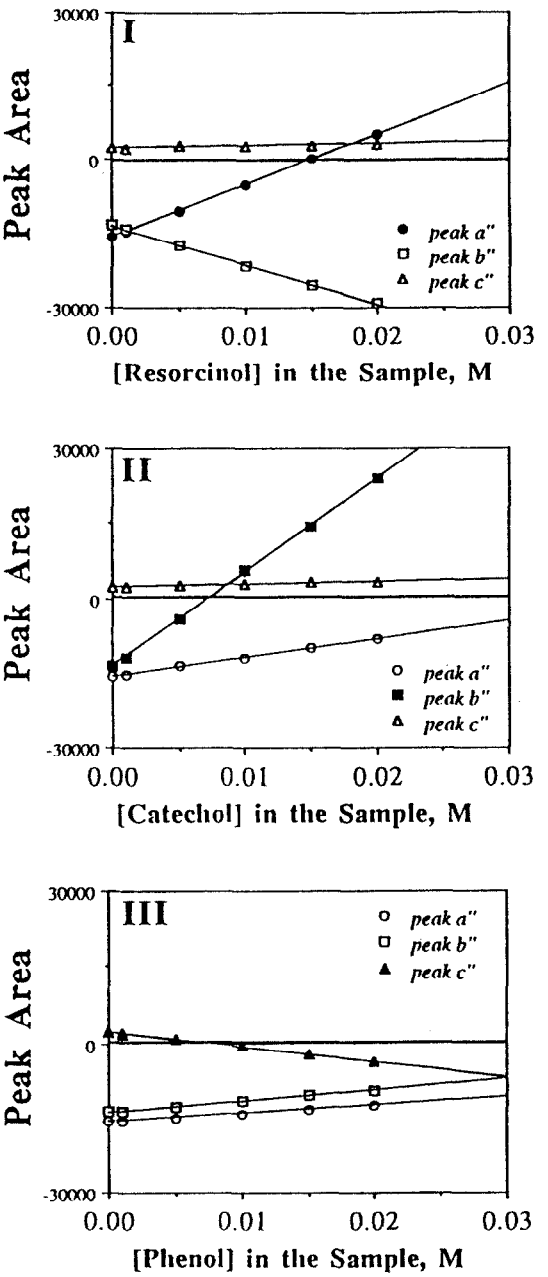

Fig. 9. Plots of the areas of the three system peaks versus the sample concentration. Sample: I, resorcinol; II, catechol; III, phenol. Mobile phase, resorcinol–catechol–phenol (10 mM each) (1:1:1). Detection at 298 nm.

decrease in the area of the third system peak, which, initially positive (for a vacancy, see Fig. 1-II, chromatogram 5), becomes negative for a

phenol concentration slightly lower than 10 m*M*. The areas of the first two peaks, initially negative, increase slowly with increasing sample size, i.e., decrease in absolute terms. The catechol concentration in the third peak decreases with increasing phenol sample size, while the catechol concentration increases in the second peak. This result is again in agreement with the system peak theory. It shows also that there is competition between phenol and the two hydroxyphenols, as phenol is not detected at 298 nm. This competition explains the decrease in the catechol content of the third system peak.

5. Conclusions

The experimental results presented above validate earlier theoretical developments regarding the behavior of system peaks in multi-component chromatographic mobile phases [1,16]. The equilibrium of the retained additives between the mobile and the stationary phases is perturbed by the injection of any sample having a composition different, however slightly, from that of the mobile phase. The study of these perturbations can provide useful information on the nature of the equilibrium. It is important to recognize that, because the equilibrium isotherms are not linear, and that they are coupled in multi-component systems, the retention times of the pulses, and also their areas, depend on the mobile phase composition, and each system peak is actually a local perturbation of all the equilibria involved.

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