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DETERMINATION OF THE PHASE RATIO BY MEANS OF REACTION KINETICS IN A LIQUID CHROMATOGRAPHIC REACTOR

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SUMMARY

The on-column rate constants in an octadecylsilane (ODS) bonded liquid chromatographic reactor with methanol mobile phase were used to determine the phase ratio and catalyst distribution coefficient. A concentration factor and the extent of ODS participation were introduced to treat the contribution of the non-polar alkyl ligands to solute retention in the composite stationary phase. The results obtained from this operational approach which can be expanded to other systems are quite comparable to literature values, and the discrepancies are discussed.

INTRODUCTION

In a previous study we used reaction rate constants to characterize an octadecylsilane (ODS) bonded stationary phase which involved hydrocarbon moieties with associated methanol¹. Use of on-column reaction kinetics to complement spectroscopic and retention studies for derivatized surface characterization is appropriate where these rate measurements are demonstrably free of mass transfer limitations in the microparticulate-packed liquid chromatographic (LC) column². Due to the nature of the composite chemically-bonded stationary phase and problems in interpreting solute retention behavior¹, phase ratio determinations in reversed-phase liquid chromatographic (RPLC) systems are elusive. The phase ratio (ϕ) is defined as V_s/V_m where V_s and V_m are stationary phase and mobile phase volumes. Since the boundary between these two phases is not well defined and subject to mobile phase composition change³⁻⁷, there has been only limited discussion on phase ratio determination. On the basis of results for using a reactant molecule as a probe to characterize the stationary phase with a methanolic mobile phase, we describe here an operational approach for determining the phase ratio and from it solute distribution coefficients in a chemically-bonded chromatographic system.

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THEORETICAL

With a first-order reaction occurring in both the mobile and stationary phases, we have shown that a composite or apparent rate constant, k_{app} , can be obtained from the decrease of the reactant peak areas (A_R) with retention time (t_R)^{1,2} in reaction chromatograms (see Fig. 1 for illustration). Thus

$$\ln A_R = \ln A_R(t=0) - k_{app} t_R \quad (1)$$

and

$$k_{app} = k_m \left(\frac{t_m}{t_R} \right) + k_s \left(\frac{t_s}{t_R} \right) \quad (2)$$

where k_i and t_i represent first-order rate constants and reactant retention time in the designated phase, mobile ($i=m$) or stationary ($i=s$). The k_m values can be measured independently in a batch reactor using the mobile phase as solvent^{8,9}; k_s is then obtained by substituting k_m into eqn. 2.

For a pseudo-first order reaction in solvent with catalyst in large excess,

$$k_m = k_{MeOH}^{(2)} [\text{catalyst}]_m \quad (3)$$

where $k_{MeOH}^{(2)}$ is the second-order rate constant in methanol ($M^{-1} s^{-1}$), and $[\text{catalyst}]$ is the 4-picoline or pyridine catalyst concentration (M^{-1}) in the designated phase.

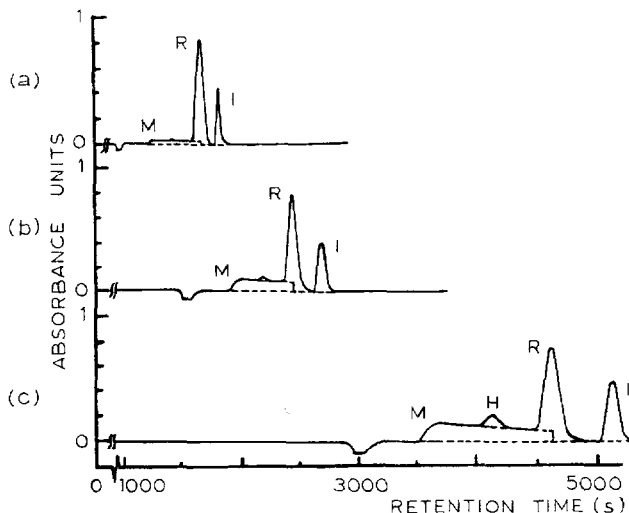


Fig. 1. Series of liquid chromatograms for TCTPCl₂ esterification reaction catalyzed by 0.0075 *M* pyridine in methanol at 25°C. Intermediate salt formation is illustrated. R is the reactant (TCTPCl₂), I the inert standard (1-phenylheptane), M the intermediate product [N-(4-(chlorocarbonyl)tetrachlorobenzoyl)pyridinium chloride], H the half ester impurity (methyl, Cl-TCTP), and C the catalyst vacancy peak (pyridine). Conditions were as follows: (a) flow-rate 0.32 ml/min, $\Delta P = 900$ p.s.i.; (b) flow-rate 0.21 ml/min, $\Delta P = 700$ p.s.i.; (c) flow-rate 0.11 ml/min, $\Delta P = 500$ p.s.i.

For the reaction in a composite stationary phase, a concentration factor (C.F.) can be utilized as a first approximation to accommodate the dilution effect of the non-polar ODS moieties in the reaction kinetics¹. (The pertinent esterification reaction here does not proceed in non-polar hydrocarbon solvent^{8,9}.) Therefore,

$$k_s = k_{\text{MeOH}}^{(2)} [\text{catalyst}]_s (\text{C.F.}) \quad (4)$$

Since the base catalyst distribution coefficient, K_{CAT} , is defined as

$$\begin{aligned} K_{\text{CAT}} &= [\text{catalyst}]_s / [\text{catalyst}]_m \\ &= (V_{\text{R,CAT}} - V_m) / V_s \end{aligned} \quad (5)$$

V_m can be determined using either the homologous series method or from the retention volume of non-retained species¹⁰. $V_{\text{R,CAT}}$ is the retention volume of the base catalyst in the column. Combining eqns. 3-5, we obtain

$$k_s = k_{\text{MeOH}}^{(2)} [\text{catalyst}]_m \frac{[\text{catalyst}]_s}{[\text{catalyst}]_m} (\text{C.F.}) = k_m K_{\text{CAT}} (\text{C.F.}) \quad (6)$$

and

$$K_{\text{CAT}} = \frac{k_s}{k_m} \frac{1}{\text{C.F.}} \quad (7)$$

Since the catalyst capacity factor, k'_c , is related to K_{CAT} and phase ratio φ :

$$k'_c = K_{\text{CAT}} \varphi \quad (8)$$

or

$$\frac{V_{\text{R,CAT}} - V_m}{V_m} = \left(\frac{V_{\text{R,CAT}} - V_m}{V_s} \right) \left(\frac{V_s}{V_m} \right) \quad (9)$$

thus,

$$k'_c = (k_s/k_m) (\text{C.F.})^{-1} \varphi \quad (10)$$

Earlier, we have shown that there is some indicated contribution of the bonded ODS ligands to solute retention through a dispersion type of mechanism¹. Here, the extent of ODS ligands participating in solute retention, ξ , is introduced to calculate the total stationary phase volume, V_s :

$$V_s = V_{s,\text{MeOH}} + \xi V_{\text{ODS}} \quad (0 \leq \xi \leq 1) \quad (11)$$

and

$$\text{C.F.} = \frac{V_{s,\text{MeOH}}}{V_{s,\text{MeOH}} + \xi V_{\text{ODS}}} \quad (12)$$

where $V_{s,\text{MeOH}}$ is the volume of associated methanol pseudo-layer determined by the adsorption isotherm experiment^{1,11}, V_{ODS} is the volume of the bonded ODS ligands. The intercalated methanol molecules might associate with the silica surface as well as with the immobilized C_{18} chains as has been suggested by others³⁻⁶. If $\xi = 0$, then C.F. is unity, which corresponds to the idealized adsorption type of model proposed by Knox and Pryde¹² and other investigators¹³⁻¹⁷. If $\xi = 1$, then $\text{C.F.} = V_{s,\text{MeOH}}/(V_{s,\text{MeOH}} + V_{\text{ODS}})$, which is the idealized dispersion or partition model discussed earlier^{1,3}.

Substituting eqn. 12 into eqns. 6 and 10, then

$$k_s = k_m K_{\text{CAT}} \frac{V_{s,\text{MeOH}}}{V_{s,\text{MeOH}} + \xi V_{\text{ODS}}} \quad (13)$$

and the phase ratio φ :

$$\begin{aligned} \varphi &= \frac{k'_c k_m}{k_s} (\text{C.F.}) \\ &= \frac{k'_c k_m}{k_s} \frac{V_{s,\text{MeOH}}}{V_{s,\text{MeOH}} + \xi V_{\text{ODS}}} \end{aligned} \quad (14)$$

The direct determination of φ then becomes possible if the following equation is introduced:

$$\varphi = \frac{V_s}{V_m} = \frac{V_{s,\text{MeOH}} + \xi V_{\text{ODS}}}{V_m} \quad (15)$$

By equating eqns. 14 and 15:

$$\frac{k'_c k_m}{k_s} \left(\frac{V_{s,\text{MeOH}}}{V_{s,\text{MeOH}} + \xi V_{\text{ODS}}} \right) = \frac{V_{s,\text{MeOH}} + \xi V_{\text{ODS}}}{V_m} \quad (16)$$

Thus,

$$\xi = \frac{1}{V_{\text{ODS}}} (\sqrt{k'_c k_m V_{s,\text{MeOH}} V_m / k_s} - V_{s,\text{MeOH}}) \quad (17)$$

Substituting eqn. 17 into eqn. 15,

$$\varphi = \sqrt{\frac{k'_c k_m}{k_s} \frac{V_{s,\text{MeOH}}}{V_m}} \quad (18)$$

With the known kinetic data in both phases, k_m and k_s , the catalyst capacity factor, k'_c , and V_m and $V_{s,\text{MeOH}}$, the phase ratio, extent of ODS participation, ξ , and C.F.

can be determined. This then represents the first attempt to quantify the solute retention in a chemically-bonded chromatographic system through results from reaction kinetic studies.

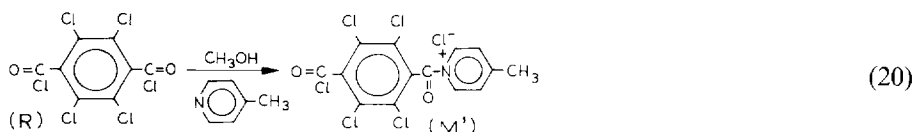
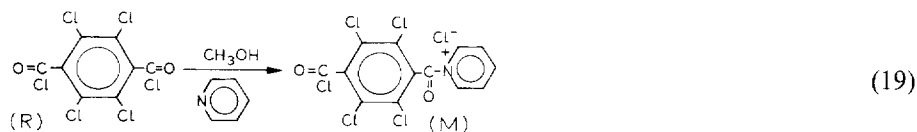
EXPERIMENTAL

Liquid chromatographic reactor system

The LC system of our laboratory employs two Altex Ultrasphere-ODS high-performance liquid chromatography (HPLC) columns in series connected with a 0.5 in. \times $\frac{1}{16}$ in. tubing (0.01 in. I.D.). Two columns were used to provide longer residence times for the relatively slow esterification reactions to proceed. With this arrangement, appreciable conversions are achieved for better kinetic measurements. The columns, 25 cm \times 4.6 mm I.D., contained 3.2 g of 5- μ m particles with a surface area of 200 m²/g and an average pore diameter of 80 Å; they were thermostated by circulating water for reaction temperature control. The bonded stationary phase was a monomolecular layer of octadecyldimethylsilane, with trimethylsilyl end capping. The surface coverage of octadecyldimethylsilane is reported as 3.0 μ mol/m² and the packing material is 12% (w/w) carbon¹⁸. The mobile phase solvent was pumped through the column with a Waters 6000A solvent delivery system. The reaction sample solutions were introduced to the solvent stream with a Beckman Series 210, four-port sample valve with a 20- μ l sample loop. The effluent passed through a Perkin-Elmer LC-55 variable-wavelength spectrophotometer detector adjusted at a detection wavelength of 223 nm. The peaks were timed and recorded with a Spectra-Physics SP4000 data processor as well as a Honeywell Electronik 194 recorder. The flow-rate was checked with a vertical 1-ml pipet, graduated in 0.01-ml increments; a two-way valve permitted diversion of the outlet stream through the pipet for flow measurement. The time between graduations was measured with a stopwatch.

Reactions and reagents

The studied reactions were pyridine or 4-picoline catalyzed esterification reactions of tetrachloroterephthaloyl chloride (TCTPCI₂, R) to form quaternary pyridinium or picolinium chloride salts (M or M'):



Distilled-in-glass grade methanol was obtained from Burdick & Jackson Labs. (Muskegon, MI, U.S.A.). The methanol solvent used as the HPLC mobile phase was further filtered through a Fluoropore filter (Millipore, Bedford, MA, U.S.A.) with a 0.5- μ m pore size. Pyridine and 4-picoline catalysts purchased from Aldrich (Mil-

waukee, WI, U.S.A.) were dried over 4-Å molecular sieves for one or two days. HPLC-grade tetrahydrofuran (THF)-UV (Burdick and Jackson Labs.) was dried over 5-Å molecular sieves for two to seven days. Commercial TCTPCl₂, m.p. 146.5–148°C, was recrystallized in our laboratories¹⁹.

The mobile phases consisted of a relatively dilute base catalyst in a methanol–tetrahydrofuran solution which was 0.25 *M* in THF. The base concentrations were (1) pyridine 0.0050 *M* at 25°C, (2) pyridine 0.0050 *M* at 35°C, (3) pyridine 0.0075 *M* at 25°C, (4) pyridine 0.0075 *M* at 35°C, (5) 4-picoline 0.00615 *M* at 25°C, (6) 4-picoline 0.00615 *M* at 35°C, (7) 4-picoline 0.0082 *M* at 25°C, and (8) 4-picoline 0.0082 *M* at 35°C.

The injections were 20 μl of a methanol solution 0.0003 *M* in TCTPCl₂ reactant, 0.01 *M* in inert standard, and approximately 0.25 *M* in THF. The TCTPCl₂ concentration was low enough to ensure pseudo-first-order kinetics, linear sorption isotherm, and minimal reaction heat effect. Either UV-sensitive *n*-phenylheptane or *n*-phenyloctane (Aldrich) was utilized as an internal standard for the pyridine or 4-picoline catalyzed reaction, respectively. Tetrahydrofuran, used to aid the dissolution of TCTPCl₂ crystals in the reaction sample mixtures, was present at such a low level that no spectrophotometric interference or special solvent effect was observed on the esterification kinetics^{8,9}.

RESULTS AND DISCUSSION

The rate constants for reactions 19 and 20 in the methanol mobile phase, k_m , were determined in a batch reactor^{8,9,21}. Using eqn. 2, the rate constants in the methanol–solvated ODS stationary phase were obtained from k_{app} values measured in the described LC reactor. Table I shows four different phase ratio calculations

TABLE I

CALCULATED PHASE RATIOS FROM THE TWO-PHASE RATE CONSTANTS OF THE TCTPCl₂ ESTERIFICATION REACTIONS IN AN LC REACTOR AT 25 AND 35°C

For two Altex Ultrasphere-ODS columns in series, $V_{ODS} = 0.5 \times 2 = 1$ ml, $V_m = 4.5$ ml, and $V_{s,MeOH} = 0.56 \times 2 = 1.12$ ml (25°C) and $0.64 \times 2 = 1.28$ ml (35°C). See ref. 1 for detailed calculations. k_s/k_m was obtained, experimentally in an LC reactor; capacity factor k'_c , phase ratio ϕ , extent of ODS participation ξ , and concentration factor (C.F.) were determined using eqns. 9, 18, 17, and 12, respectively.

Catalyst	Conc. (<i>M</i>)	k_s/k_m	k'_c	ϕ	ξ	C.F.
At 25°C						
4-Picoline	0.00615	0.28 ± 0.03	0.17 ± 0.04	1/(2.7 ± 0.1)	0.59	0.65
4-Picoline	0.0082	0.32 ± 0.04				
Pyridine	0.0050	0.26 ± 0.06	0.13 ± 0.03	1/(2.9 ± 0.1)	0.48	0.70
Pyridine	0.0075	0.26 ± 0.06				
At 35°C						
4-Picoline	0.00615	0.33 ± 0.04	0.18 ± 0.04	1/(2.6 ± 0.1)	0.44	0.74
4-Picoline	0.0082	0.37 ± 0.04				
Pyridine	0.0050	0.30 ± 0.06	0.16 ± 0.04	1/(2.8 ± 0.2)	0.34	0.79
Pyridine	0.0075	0.41 ± 0.08				

from eqn. 18 with one standard deviation of error. Despite experimental errors from the reaction kinetics, retention and mobile phase volume measurements, these values are fairly consistent for different catalyst solutes at two different concentrations ($1/2.7 = 0.37$ at 35°C and $1/2.8 = 0.36$ at 25°C). The consistency suggests a linear isotherm free of concentration dependence at catalyst concentrations up to $10^{-2} M$. It appears that the C.F. values are between 0.65 and 0.79 because not all ODS ligands are participating in the solute retention ($0.34 \leq \xi \leq 0.59$) as a consequence of steric and geometric effects.

Thus, use of a reactant molecule in a defined reaction system can provide an operational new approach to the determination of the "effective" or "dynamic" phase ratio. This or a related approach is particularly attractive because of the difficulty in obtaining the phase ratio from direct V_s/V_m measurement, a consequence of the ill-defined boundary between the two phases in RPLC. Another approach using the carbon load and silica surface area is less satisfactory because surface derivatization can significantly reduce surface area due to the narrowing and/or blocking of silica pores by the bonded ligands^{22,23}. With the stationary phase in RPLC no longer a "static" entity, variation of the phase ratio with the type and composition of organic modifier in the mobile phase further complicates the problem^{3-6,24}.

The φ values measured by the reaction kinetic method are comparable to the $1/2.6$ value reported for the μ Bondapak C₁₈ column²⁵; however, no detail was presented as to how this value was calculated using the manufacturer's data on silanol surface coverage and percent carbon loading. Yonker *et al.*³ also obtained a $1/2.3$ phase ratio at 100% methanol in the LiChrosorb RP-18 column at 25°C from the total stationary volume divided by the mobile phase volume. Their determination appears to be close to estimates ($1/1.8$ – $1/2.2$) if one assumes the concentration factor equal to one [*i.e.* $\varphi = k'_c(k_m/k_s) = k'_c/K_{\text{CAT}}$]. This is reasonable since they assumed the total stationary phase volume to be the solvation layer volume only without considering the bonded octadecylsilane volume which may also be responsible for solute retention. While the agreement between phase ratio values determined by the kinetic method here and those determined by other cited methods is good, it seems desirable to carry out measurements with other irreversible reactions. Further agreement between measurements made with different reactants on a given column is required (in the future) if the approach described here is to be used with any degree of confidence to determine the phase ratio as a physical parameter of a chromatographic column.

It also is worth noting that the phase ratio here does not change significantly with temperature increase. Although thermal energy may slightly increase ligand unfolding so that more eluent molecules can associate onto the ligand surfaces^{26,27}, such unfolding has a tendency to shift the retention mechanism toward an adsorption type of behavior for which direct ODS participation in solute retention decreases correspondingly¹. This can be verified by the ξ value decrease from 0.48–0.59 at 25°C to 0.34–0.44 at 35°C (Table I). The immobilization of bonded alkyl moieties on the silica support also inhibits the "solubilization" of the stationary phase into the mobile phase. This phenomenon apparently would be different from the observation in a liquid–liquid partition system²⁸ that the phase ratio decreased with increased temperature.

Because of the intrinsic, inhomogeneous composition in the stationary phase,

the phase ratio measurement with correction for the concentration factor and extent of ODS participation may well reflect the nature of the chromatographic matrix for retention of solute and its interactions with the composite stationary phase. Conceptually, the stationary phase consists of the associated solvent layer sheathed on the surfaces of residual silanol groups as well as the bonded octadecylsilane moieties. The kinetic approach circumvents the ambiguity of designation of adsorption *versus* partition or a mixed mechanism, and permits a view of dynamic two-phase interactions. The inclusion of the "inert" hydrocarbonaceous monolayer (as assumed in the hydrophobic or solvophobic theory) in quantifying the phase ratio is especially important for the LC systems with bonded long-chain hydrocarbons in an organic-rich environment where the ligands are fully solvated by the organic solvent²⁹⁻³². Furthermore, for the adsorption type, non-derivatized silica packings or short-chain alkyl bonded systems (*i.e.* low carbon loadings), the extent of alkyl participation can be insignificant ($\xi \rightarrow 0$), then, eqn. 10 can be used to determine the phase ratio directly assuming the concentration factor is unity. This is because mobile phase solvent molecules will be adsorbed onto the silica surface and form a stagnant pseudo-layer of related solvent nature^{7,13-17}. This might also be true for long-chain hydrocarbon-bonded systems in a water-rich environment where the ligands are preferentially "collapsed" on the silica surface due to their hydrophobic nature, thus reducing their contribution to solute retention.

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