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Review

Reaction kinetics and kinetic processes in modern liquid chromatographic reactors

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ABSTRACT

The analysis and use of liquid chromatographic columns as chemical reactors are explained. Multifold applications of liquid chromatographic reactors with a wide range of materials are described and illustrated. Means for extracting chemical kinetic rate data from liquid chromatographic reactor experiments in the presence of other dynamic processes are emphasized. Techniques for controlling reactivity and selectivity are explained. It is also shown that useful information about the stationary phase and protein interaction with the stationary phase can be accessible from these experiments. Several explanations for multiple zone formation including results from interconversion studies are presented. It appears that a broader awareness of chromatographic reactor applications would be beneficial to workers in a variety of different fields. There are a number of additional possibilities for the future including the removal and detoxification through transformation of environmentally undesirable materials.

CONTENTS

1. Introduction . . . 2
2. Theory . . . 4
   2.1. The ideal chromatographic reactor (ICR) . . . 6
   2.2. Modifications of ICR assumptions . . . 8
3. Chemical kinetic applications . . . 11
   3.1. Liquid–solid (adsorption) chromatographic reactors . . . 13
   3.2. Bonded-phase liquid chromatographic reactors . . . 15
   3.3. Interconversions and fast equilibria studies . . . 17
4. Kinetic processes related to LCR applications . . . 20
   4.1. Dynamic processes leading to non-ideal behavior . . . 20
   4.2. Association–dissociation kinetics and affinity chromatography . . . 21
5. Special approaches and applications . . . 22
   5.1. Characterization of column beds . . . 23
   5.2. Modifications of reactivity and selectivity . . . 24
   5.3. Large-scale application . . . 26
6. Conclusions . . . 27
7. Acknowledgements . . . 27
References . . . 27

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1. INTRODUCTION

During the past 15 years, liquid chromatography has emerged as one of the most powerful techniques available for the separation, identification and production of chemicals because of its versatility and freedom from the volatility requirement of gas chromatography. The merits of speed, efficiency, accuracy and ease of use have permitted modern liquid chromatography to be applied to compounds that range from simple organic species to complex macromolecular proteins. It was, however, the appreciation of similarities as well as differences between gas and liquid chromatography and the evolving understanding of gas chromatographic phenomena by a number of investigators that laid the foundation for the development of modern liquid chromatography. Much of the understanding was achieved through the analysis and interpretation of the physico-chemical components of chromatographic processes together with recognition of their dynamic character. Chromatographic equipment or procedures were altered so that information on the thermodynamics of solutions, gas–solid interactions, adsorption isotherms, diffusion and mass transfer processes all became available from gas chromatograms. It is now generally recognized that where applicable and suitable equipment is accessible, gas chromatographic techniques are among the most versatile for studying these phenomena [1–3].

Physico-chemical information can also be obtained from liquid chromatography although there are more complications from solution phenomena associated with the stationary phase, interaction between the mobile phase and the stationary phase and a wide assortment of solute–mobile phase interactions. Thus, liquid chromatographic processes are more complex than those of gas chromatographic systems and there have been stimulating disagreements about details of theory and the interpretation of pertinent physico-chemical phenomena [4–8]. Hence, some of the complications and their emphasis under selected experimental conditions provide an opportunity to study physico-chemical phenomena on surfaces, in the mobile phase and in the immobilized stationary phase [1,9,10].

The interpretation of the response to a pulse input in a catalytic microreactor equipped with a separate analytical chromatograph and similar equipment for kinetic and surface investigations has been common and extremely useful since the early days of gas chromatography [11,12]. However, the application of the column as a combined chemical reactor and separation device for studying and carrying out reactions has been utilized less, although there has been continuing, increased interest. This application is illustrated in Fig. 1, where a representative reaction chromatogram for a pulse of volatile reactant (cyclopentadiene dimer) dissociating to a more volatile product (cyclopentadiene) is shown. The relative positions of the reactant and product peaks would be reversed where the product is retained longer than the reactant. The column performs both reactor and chromatographic functions.

Earlier applications involving gas chromatographic systems led to several discussions of the advantages of chromatographic reactors relative to conventional static and flow reactors [2,3,13]. The attractive features of using chromatographic columns as chemical reactors with continuous mobile phase flow include the following: (a) the presence of concerted separation and reaction processes throughout the column; (b) the possibility of exceeding equilibrium conversions for reversible reactions as well as selective production of intermediate species with high purity in series reactions; (c) the capacity for quantitatively handling products and reactants readily without problems of transfer; (d) the ease of measuring the amount of reactant

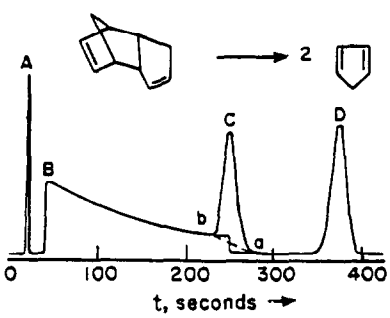

Fig. 1. Reaction chromatogram for the dissociation of dicyclopentadiene to cyclopentadiene. Column, 25% Apiezon L, 10 ft. × 0.25 in. I.D. at 209.6°C. A, air; B, cyclopentadiene; C, dicyclopentadiene reactant; D, inert standard. Carrier gas, helium. The line ba represents product formed during elution of the reactant peak.

introduced to the column and eluted from it and determining residence times in both the stationary phase and the mobile phase during passage through the column for each species; and (e) the ease of manipulation and control of chromatographic apparatus and associated detectors. Also, transport and sorption process rates frequently exceed those of chemical kinetic processes with gas chromatography so that only reaction rate is limiting and other effects frequently can be ignored.

While the possibility of carrying out reactions to advantage in gas chromatographic columns has been well realized in many instances [12–15], reaction kinetic studies and applications in liquid chromatographic columns have been less developed despite a number of incentives which can be envisioned for the future. In addition to the less ideal behavior inherent in the liquid, another factor is that many time-dependent processes such as mass transfer and sorption–desorption are much slower in the liquid phase than in the gas phase. This leads to complications in studying chemical reactions of comparable or faster rates because an additional category of kinetic processes must be considered. Since these relevant processes are slower, the chemical reactions studied in the column should also be slower in the best situations. Sometimes, modified chromatographic procedures can be invoked to compensate for slow chemical reaction, as will be illustrated later.

Until recently, many facets of liquid chromatographic reactors (LCR) have been unexploited although they have been gaining recognition [16–24]. However, much of the potential and many advantages of these systems are unrealized and unrecognized by many, awaiting further demonstration and investigation. Thus, a number of aspects of liquid chromatographic reactors and associated phenomena are appropriate for further study aided by continuing instrumental and theoretical developments. This is not to imply, however, that many of the virtues of liquid chromatographic reactor operations have not been discussed or utilized. Possibilities for exceeding equilibrium conversions through the elimination of reverse reactions by separation of products have been recognized and demonstrated by Wetherold *et al.* [25], Cho *et al.* [26,27] and Villermaux and co-workers [28,29]. Although application of the column as a reactor for preparative purposes has been infrequent, this potential is now being recognized [30–33]. On a much larger scale, Deans and co-workers [34,35] have even utilized ethyl acetate hydrolysis as a probe reaction in a subterranean oil reservoir together with chromatographic processes to obtain information on the reservoir bed. One major limitation for chromatographic reactor operations was the requirement that reactions proceed sufficiently fast for significant concentration changes during column residence. A means of compensating for slow reaction kinetics to provide for adequate changes is through "stopped flow", which can extend the range of kinetic studies in some instances [36–39]. A related technique involves immobilization on the surface followed by dissolution reactions, as explained later [40,41]. Pre- and post-column derivatization techniques for analysis and separation are, of course, well advanced and are discussed elsewhere [42–44].

With understanding and appreciation of liquid chromatographic reactor features, more opportunities for broader applications of modern liquid chromatographic systems should emerge, especially in environmental and biochemical related areas. For instance, one can ask whether or not there are possibilities for selectively reacting and removing small amounts of materials which are of environmental interest [45], whether reaction kinetic studies can assist the interpretation of chromatograms when some inadvertent biochemical reaction occurs [18,19,24] or the way in which interconversion reactions affect separations [20–23,46–48]. The purpose of this paper is to review recent work on liquid chromatographic reactor applications and related kinetics and, it is hoped, to provide some insights into the potential and limitations of LCR operations for other chromatographic investigators. An attempt is also made to show how common features and approaches have arisen among workers in diverse areas in hope that this recognition will benefit all. Because of space limitations the discussion has to be illustrative rather than comprehensive, although a broad range of topics and related literature are included together with leading references. The following section covers some fundamental principles of liquid chromatographic reactors and some strategy for handling models. LCR applications in chemical kinetics with chromatographic separation interactions are discussed in Section 3.

Section 4 presents a selection of recent studies on dynamic processes related to chemical reactions occurring in liquid chromatographic columns. Finally, some promising facets and developing potential applications of the LCR are discussed in Section 5.

2. THEORY

The chromatographic reactor of special interest here can be characterized as a chromatographic column–reactor system into which a reactant solute or reaction mixture is introduced and subsequently converted to products in the course of passage through the column. The hypothetical chromatogram in Fig. 2 resulting from a reactant pulse illustrates the behavior and consequences of concerted reaction, partitioning and separation in the column. Reactant and product materials are distributed between the mobile and stationary phases in a manner governed by characteristic partition coefficients. Where partition coefficients for reactants and products differ, separation occurs. Different types of chromatographic columns could be adapted for reactor use with either gas or liquid mobile phases; the stationary phase can be liquid coated on a solid support, one molecularly attached to the support or an adsorbent solid. Under proper conditions, reactant concentration changes and kinetic rate constants can be determined [13,47]. The ordinary chromatographic process results in constant dilution of eluites; thus, with the inherent presence of longitudinal diffusion and a significant pressure gradient (with gas chromatography), kinetic studies of first-order and pseudo-first-order reactions are most straightforward. However, special complex systems can be considered. For preparative applications and qualitative characterization purposes, requirements can be less stringent.

Many treatments of theory relevant to liquid chromatographic reactors are derived from the broad applications of gas chromatographic columns as chemical reactors. Gas chromatographic reactor behavior mainly involves a single-phase surface or two-phase homogeneous reaction in the case of heterogeneous catalytic reactor beds and gas–liquid partition columns, respectively. Analogous in the liquid chromatographic reactor are reaction systems in liquid–solid adsorption chromatography, and liquid–liquid partition or bonded-phase chromatography. With early theoretical treatments having focused on gas chromatographic reactors and related kinetic processes, a number of reviews are available with extensive coverage of these developments [12–15,49–51]. Although many concepts are not directly transferable, these studies have helped accelerate some understanding and more recent developments of liquid chromatographic reactors.

While there were discussions of mass transfer kinetic effects on band broadening and column efficiency in the early stages of modern liquid chromatographic development [52–56], studies of kinetic processes coupled with chemical reactions were limited. Recently, additional models of various complexities applicable to LCR kinetic studies have emerged [20,24,57–60]. Because of the complexity of the concerted reaction and separation processes in the chromatographic reactor, a number of problems remain, *e.g.*, solutions when all rate processes in both phases are considered, situations involving reactions of higher orders, and treatments involving stationary phase inhomogeneity.

Affinity chromatography has been among the popular liquid chromatographic techniques where considerable attention has been directed toward kinetic processes in the column because of the underlying chemical nature of interactions between solutes and ligands. Strictly, this type of system does not fall into the "chromatographic reactor" category as defined here, as no net chemical changes occur to the eluite between entering and leaving the column. Therefore, despite the increasing importance of affinity chromatography especially in biotechnology areas, it receives limited attention here with brief summaries of some important work in Section 4. Readers interested in the details of kinetic models describing affinity chromatography can refer to other reviews [61,62].

To provide a basis and perspective for discussing recent LCR developments, a chronological list of some of the significant progress of the past is presented by way of introduction. In Table 1, some important modelling efforts during the early development of gas chromatographic reactors are summarized, and in Table 2, some theoretical treatments more directly related to liquid chromatographic reactors are listed. In the section below, the limiting ideal reactor model is introduced first, followed by a

TABLE 1

SOME MODELS FOR TREATING REACTIONS IN GAS CHROMATOGRAPHIC REACTORS

Reaction	Reaction phase(s)	Linearity of isotherm	Ideality assumption[a]	Solution	Ref.
A→B 1st irrev.	Stationary	Yes	No	Plate theory	63
A⇌B 1st rev.	Stationary	Yes	Yes	Number of ideal stages for reaction	64
A→B 1st irrev.	Gas and surface	Yes	A/D	Continuous-flow model	65
*n*th-order irrev.	Surface	Yes	Yes	Input pulse shape effect	66
A→B 1st irrev.	Gas and surface	Yes	AX	Continuous-flow model; limiting case	67
A→B 1st irrev.	Surface	Langmuir	A/D	Continuous-flow model	68
A→B 1st irrev.	Gas and surface	Yes	Yes	Continuous-flow model (ICR)	47
Multi-comp.	Stationary	No	MT	Moment analysis	69
A+B⇌2C 2nd rev.	Stationary	Yes	No	Plate model	70
A→B+C 1st irrev.	Surface	Yes	A/D	Moment analysis	71
A⇌B+C 1st and 2nd rev.	Surface	Langmuir	AX, A/D	Continuous-flow model	72
A→C or A+B→C 1st irrev.	Surface	Yes	AX, MT, A/D	Moment analysis	73
A→B 1st irrev.	Surface	Langmuir	AX, A/D	Moving bed	74
*n*th-order	Surface	Power law and Langmuir	Yes	Input pulse shape effect	75
A⇌B 1st rev.	Apparent for column	Yes	No	Plate theory	76
A⇌B 1st rev.	Apparent for column	No	No	Empirical equation for asymmetric peak	77
A→B 1st irev.	Surface	Yes	MT, A/D	Moment analysis	78
A→B→C 1st consec.	Surface	Yes	Yes	Moving bed	79
A⇌B+C	Apparent for column	Yes	No	Plate model	57
A→B 1st irrev.	Gas and surface	Yes	AX, MT	Moment analysis	80
A⇌B 1st rev.	Stationary	Yes	No	Plate model	81
A→B 1st irrev.	Stationary	Yes	AX, MT	Moment analysis	82
A⇌B 1st rev.	Surface	Langmuir	AX, A/D	Moving bed	83, 84

[a] A/D = Adsorption–desorption kinetics; AX = axial dispersion included; MT = interphase mass transfer or stationary phase (intraparticle) diffusion.

TABLE 2

KINETIC MODELS APPLICABLE TO LIQUID CHROMATOGRAPHIC REACTORS

Reaction	Column type	Reaction phase(s)[a]	Linearity	Ideality[b]	Approach	Ref.
$A \rightleftharpoons B$	General	Apparent	Yes	AX	Random walk model	46
$A \rightarrow B$	General	Both	Yes	AX, MT	Moment analysis	85
$A \rightleftharpoons B + C$	LC	Apparent	Freundlich	AX	Finite difference	25
$A + S \rightleftharpoons A \cdot S$	General	Stationary	Yes	AX, A/D	Steepest descent approximation	86, 87
$A + L \rightleftharpoons AL$	Affinity	Stationary	Yes	No	Moment analysis	88
Multi-comp. 1st irrev.	General	Both	Yes	AX, MT	Moment analysis	89
$A + B \rightleftharpoons AB$	LC	Mobile	Yes	MT, A/D	Moment analysis	90
$A + L \rightleftharpoons AL$	LC	Stationary	Yes	No	Plate model	56
General	General	Apparent	No	Yes	Vanishing species theorem	29
$A \rightleftharpoons B + C$	LC	Apparent	Freundlich	AX	Finite difference	26, 27
$A + L \rightleftharpoons AL$	Affinity	Both	Yes	No	Moment analysis	91
$A \rightarrow B$	LC	Surface	Yes	No	Plate model	92
$A \rightleftharpoons B$	LC	Both	Yes	Yes	Moment analysis	20, 93
Multi-comp. 1st rev.	LC	Both	Yes	No	Probability model	24
$A + B \rightarrow C$	LC	Both	Yes	AX, MT, A/D	Moment analysis	58
$A + L \rightleftharpoons AL$	Affinity	Stationary	No	No	Thomas theory	94
$A \rightarrow R \rightarrow S$	General	Both	Langmuir	No	Selectivity simulation	95
Multi-comp. 1st irrev.	General	Both	Yes	AX, MT	Fast Fourier transform	60
$A \rightarrow B$	LC	Surface	Yes	A/D	Moment analysis	59

[a] Apparent = Apparent (mobile and stationary) rate for column.
[b] See Table 1 for abbreviations.

discussion of some modifications which permit real operations to be described and a few case studies.

2.1. The ideal chromatographic reactor (ICR)

For understanding the features and advantages of chromatographic reactors, the limiting "ideal chromatographic reactor" (ICR) situation can be reviewed as a basis for comparison with conventional plug-flow (PFR) and continuous stirred-tank reactors (CSTR) [13,47]. Briefly, in such an ideal column reactor–separator:

(1) A reacting pulse is swept through the column with instantaneous separation from resulting products. The reaction and instant separation feature contrasts with the concept of instant mixing in the CSTR, and also differs from the uniform flow pattern in the PFR.

(2) The column is homogeneous in composition with respect to both the mobile phase and the stationary phase. In liquid chromatography, this treatment of the mobile phase is particularly valid, as the fluid is essentially incompressible.

(3) Distribution isotherms are linear, *i.e.*, the partition coefficients between the mobile and stationary phases for all reactants and products are independent of concentration.

(4) Peak spreading and axial dispersion are not important in the ideal case. For reactions other than first order, these effects must be minimal.

(5) The conversion process is controlled by chemical reaction, so that mass transfer and adsorption–desorption rates in the column are fast relative to chemical reaction rate.

(6) The column is isothermal; heats of reaction and solution are negligible with the types of small samples under consideration.

The simplified general material balance on a differential section of a chromatographic column

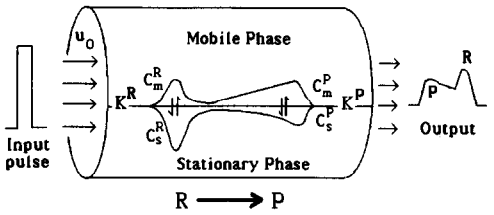

Fig. 2. Illustration of reactor chromatogram formation from concerted reaction and separation processes in a chromatographic column. K^R and K^P are the partition coefficients for the reactant (R) and the product (P). C_m and C_s represent concentrations in the mobile (m) and stationary (s) phases for the designated species [96].

with constant mobile phase velocity (Fig. 2) thus becomes

$$f_m\left(\frac{\partial C_m}{\partial t}\right) + f_s\left(\frac{\partial C_s}{\partial t}\right) = -u_0 f_m\left(\frac{\partial C_m}{\partial x}\right) - f_m r_m - f_s r_s \quad (1)$$

accumulation of reactant = net change of reactant due to flow + change in reactant due to chemical reaction in mobile and stationary phases

where

C_m, C_s = concentration of reactant in the mobile and stationary phase, respectively;
f_m, f_s = volume fraction of the mobile and stationary phase, respectively (for adsorption-type columns, the porosity ε can be used instead);
r_m, r_s = rate of reactant depletion in the mobile and stationary phase, respectively;
u_0 = linear velocity of the mobile phase;
t = time measured from injection;
x = position in the column measured from inlet.

From this equation, for first-order reactions with linear isotherms in a chromatographic column it is readily shown that [13,47]

$$\frac{W_{out}}{W_{in}} = \exp[-(k_m t_m + k_s t_s)] \quad (2)$$

where W_{in} and W_{out} are the total amounts of reactant entering and leaving the column, k_m and k_s are forward reaction rate constants in the mobile and stationary phase, respectively, and t_m and t_s are the corresponding residence times for the reactant.

Methods for determining reaction rate constants in chromatographic reactors have been reviewed [13,15]. Among them, the "inert standard" method is commonly used. If a known amount (W) of inert reference material (I) is added to a reactant (R) mixture before injection, then

$$\ln\left[\frac{A_R}{A_I}\right] = \ln\left(\frac{W_{R,in}/S_R}{W_I/S_I}\right) - k_{app} t_R \quad (3)$$

and

$$k_{app} \equiv \left(\frac{t_m}{t_R}\right) k_m + \left(\frac{t_s}{t_R}\right) k_s \quad (4)$$

where k_{app} is the apparent or composite rate constant in the column, t_R is the total column residence time for the reactant, A represents peak area and S represents detector sensitivity. With A_R/A_I and t_R measured experimentally from reactor chromatograms at varying flow-rates (Fig. 3), k_{app} can be evaluated.

It should be noted that k_m and k_s are the conventional kinetic rate constants in the mobile and stationary phase, respectively, not k_{app}. By

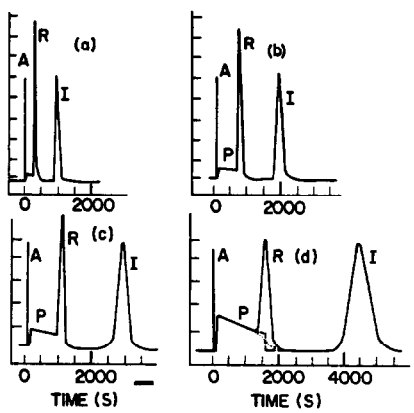

Fig. 3. Inert standard method for determining rate constants using gas chromatography. A methylcyclopentadiene dimer dissociating at 180.08°C in hexatriacontane–Gas Chrom Q (20:80), 122 cm × 0.48 cm I.D. column. A, air; P, product; R, reactant; I, inert (phenyl ether). (a) 15% conversion, flow-rate = 65.7 cm^3/min (corrected); (b) 25% conversion, flow-rate = 33.5 cm^3/min (corrected); (c) 34% conversion, flow-rate = 22.05 cm^3/min (corrected); (d) 50% conversion, flow-rate = 15.6 cm^3/min (corrected). (From ref. 126; reproduced with permission.)

measuring k_m in a batch reactor and k_{app} from the liquid chromatographic reactor, k_s can be evaluated with eqn. 4. Earlier investigations in gas–liquid systems were simplified by treatments of both phases as bulk fluids with the gas phase approximated as ideal [97]. For surface-catalyzed reactions, the reaction rate in the mobile phase is usually insignificant and can be neglected. In bonded-phase liquid chromatography, the stationary phase composition can be more complex and reaction kinetics in both phases can be studied through changes in experimental conditions [16,17,98].

ICR behavior can be approached experimentally by (a) using small, narrow pulses of reactant samples so that the isotherm linearity assumption is applicable, (b) utilizing slow flow-rates through the column to allow equilibrium to be established between two phases (however, for slow flow-rates the effects of axial diffusion can become more pronounced), (c) deliberately choosing a slow reaction to minimize possible complications from transport and adsorption processes, (d) selecting columns for study with packings of fine particles so that mass transfer effects can be neglected and (e) ensuring good temperature control for the entire column. Although some of the above considerations are not practical for large-scale operations, the ideal chromatographic reactor concept serves as a useful model for guiding and comparing reactor operations and provides a simple physical picture of the reactor system. With attention to the basic principles above, real reactors can be operated with features approaching those of the ICR where desired.

2.2. *Modifications of ICR assumptions*

Several modifications to the ideal reactor model have been made to accommodate some complications that affect real chromatographic systems. Non-equilibrium effects from the slow adsorption–desorption kinetics to compete with the reaction kinetics have been discussed [65,71,86,87]. Complications of chromatographic reactors with finite mass transfer rates involving longitudinal dispersion, intraparticle or stationary-phase diffusion and interfacial mass transfer between two phases also have received much attention [73,78,80,85,90]. In liquid chromatographic reactors, longitudinal dispersion is less serious than with gas chromatography because of low liquid diffusivity rates; however, this also can result in mass transfer and pore diffusion resistance. One attractive technique that can accommodate deviations from ideal chromatographic reactor behavior resulting from slow kinetic processes (*e.g.*, adsorption–desorption) is the stopped-flow method [36–39]. Isotherm non-linearity, which can be of special importance in adsorption chromatography and large-scale operations, results in another deviation from the ideal chromatographic reactor assumption [68,72,74,75,83,84].

The "discontinuous plate model" [99,100] and the "continuous flow model" [101,102] are two extremes frequently used to treat chromatographic dynamic processes. Comparisons between these two are shown in Table 3. While the plate model is generally simple and easy to use, plate-height values lose physical significance and become difficult to determine experimentally when chemical reactions confound

TABLE 3

COMPARISON OF THE CONTINUOUS-FLOW MODEL WITH THE PLATE (MIXING CELL) MODEL FOR CHROMATOGRAPHIC REACTORS

Treatment	Continuous-flow model	Plate (mixing cell) model
Description	Treat flow as continuous	Assume all processes occur stagewise (discontinuous)
Peak spreading	Introduce several physical parameters to describe spreading	Inherent spreading in treatment through introduction of the number of plates
System equations	A set of partial differential equations	A set of ordinary differential equations for each plate
Isotherm	Several types of non-linear isotherms can be incorporated	No satisfactory general expression incorporating non-linear effects
General solution	Analytical solutions difficult without simplifying assumptions	Plate number (N) used as the index parameter
First-order kinetics [13]	Ideal chromatographic reactor: $\frac{W_{in}}{W_{out}} = \exp(k_m t_m + k_s t_s)$	$N[1-(W_{out}/W_{in})]^{1/N} = k_m t_m + k_s t_s$ As $N \rightarrow \infty$, $\ln(W_{in}/W_{out}) = k_m t_m + k_s t_s$

other dynamic chromatographic processes. Therefore, solutions based on the continuous model are emphasized in this paper. A number of treatments emphasizing various aspects and complexities of reaction and kinetic processes and methods for solving the equations of proposed systems are summarized below.

2.2.1. Mathematical model. A general mathematical model for the LCR is employed here with consideration of axial dispersion (coefficient D_m), intraparticle or stationary phase diffusion (coefficient D_s) and adsorption–desorption processes (rate constants k_a and k_d) together with the reaction process [58]. A schematic representation of the rate processes considered in this model is shown in Fig. 4. Assuming first-order reactions in both phases, then for the reactant

$$f_m\left(\frac{\partial C_m}{\partial t}\right) + f_s\left(\frac{\partial \bar{C}_s}{\partial t}\right) = f_m\mathscr{D}_m\left(\frac{\partial^2 C_m}{\partial x^2}\right) - f_m\left\{\frac{\partial}{\partial x}[u(x)C_m]\right\} - f_m k_m C_m - f_s k_s \bar{C}_s \quad (5)$$

where $\bar{C}_s$ is the average reactant stationary phase concentration over a macroporous particle and $u(x)$ is the linear velocity of the mobile phase at position x in the column. The terms on the right-hand side include longitudinal diffusion, convection and reaction rate effects.

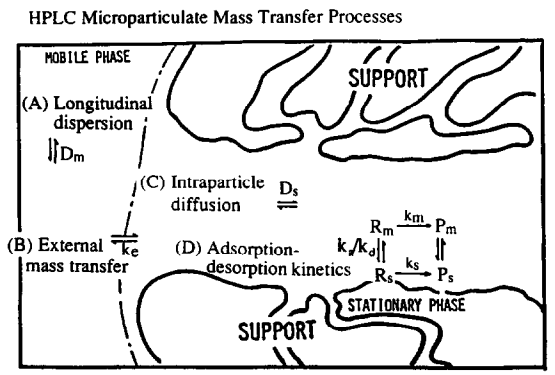

Fig. 4. Schematic representations of simple rate processes occurring in a section of a liquid chromatographic reactor. k_m and k_s, reaction rate constants in mobile and stationary phases; k_a and k_d, adsorption–desorption rate constants between mobile phase and stationary phase; k_e, mass transfer coefficient at the particle boundary; D_m and D_s, effective diffusion coefficients in mobile phase and pore [96].

For a spherical particle of packing in which reaction occurs [58],

$$\frac{\partial C_s}{\partial t} = \mathscr{D}_s\left(\frac{\partial^2 C_s}{\partial r^2} + \frac{2}{r}\cdot\frac{\partial C_s}{\partial r}\right) - k_s C_s \quad (6)$$

Here $\mathscr{D}_s$ is constant at low solute concentrations. For interfacial mass transfer in a dilute system,

$$\frac{\partial \bar{C}_s}{\partial t} = k_a C_m - (k_d + k_s)\bar{C}_s \quad (7)$$

For the model where radial uniformity is assumed, $\bar{C}_s$ is employed in eqns. 5 and 7 to eliminate any radial dependence of the $C_s(x,r,t)$ term used in eqn. 6. More complicated mathematical models dealing with stationary phase particle porosity and situations where external mass transfer across the particle boundary are important are also available [50,73].

2.2.2. Solution. With appropriate simplifications or modifications, this system can be solved or approximated using the following approaches:

(1) The ideal chromatographic reactor: by neglecting all diffusional and mass transfer resistances and assuming equilibrium distribution between two phases, the model simplifies to first-order differential equations (*e.g.*, eqn. 1) which can be solved analytically as shown previously [13,47,64,103].

(2) When the kinetics due to sorption–desorption are commensurate with chemical reaction rates, *i.e.*, distribution equilibrium is not established but all other ideal characteristics described above apply, then a solution can be obtained analytically [58,65] or statistically [71,86,87].

(3) When mass transfer processes are considered, then an analytical solution is difficult to obtain but a statistical moment approach [73,78,80,85,90] or a numerical solution [60,72,74] may be viable.

(4) Where non-linear equilibrium distributions are encountered, numerical methods usually have been utilized to solve particular situations such as those involving Langmuir and Freundlich isotherms [25–27,68,72,74,75].

(5) By varying the shapes and cycling periods of input pulses to screen and analyze responses, reactions other than first order can be studied; some kinetic parameters have been obtained for these circumstances with numerical methods [57,66,72,95, 104]. Continuous-flow feed operations in chromatographic reactors have also been studied in combi-

nation with additional parameters such as feed rate and feed position [26,27,74,78,83,84].

(6) By means of a theoretical plate treatment: this approach uses the number of plates as an adjustable parameter to incorporate all chromatographic processes that cause band broadening (Table 3). There is no direct clarification of the details of specific kinetic mechanisms for complex processes in the column. With the aid of rate theory which incorporates the contribution from each mechanism to the plate height, some kinetic parameters can be determined [63,70,76,81]. The use of a stirred tanks-in-series model with consideration of interactions at the local level also has been advocated by Villermaux to treat linear chromatography [105], with the claim that the complex phenomena involved can be represented by simple engineering concepts. Extensions to chemical reactions in both the mobile and stationary phases were illustrated.

(7) Most analyses above are based on information about reactant species only. Sometimes, it is difficult to identify the reactant peak within the overlapping elution curve which includes contributions from product concentration profiles (see Fig. 1). Then, general approaches dealing with overall elution profiles can be invoked. The statistical moment method has been applied both to the ideal situation [20,89,93,106] and some situations where non-ideal processes become important [59,82]. Alternatively, the random walk (stochastic probability) model has been utilized to treat multiple zones which appear because of interconverting species [24,46,107,108].

To illustrate and supplement some of the approaches described above, three case studies are considered below. However, the discussion is limited to a few principles and some simple results to provide an overview. Readers interested in the more detailed and involved mathematical treatments of chromatographic reactor modeling should consult the references of Tables 1 and 2.

2.2.3. Case 1. One of the most common deviations from ICR behavior stems from finite sorption kinetics for the reactant. For eqn. 7, adsorption and desorption rates are commensurate with chemical reaction rates. With a negligible dispersion effect in liquid columns, the second-order derivative term of eqn. 5 can be dropped so that a simple solution can be obtained by using proper initial and boundary conditions. For instance,

$$\text{at } x = 0, \quad C_m(0,t) = \phi(t) \tag{8}$$

$$\text{at } x = \infty, \quad C_m(\infty,t) = 0 \tag{9}$$

$$\text{at } t = 0, \quad C_m(x,0) = C_s(x,0) = 0 \tag{10}$$

where $\phi(t)$ is the reactant input function at the column inlet.

For pulse injections of reactant, the system can be solved by Laplace transformation together with the statistical moment method [58]. An expression similar to that of eqn. 3 is obtained when the inert standard method is used. However, then

$$\ln\left(\frac{A_R}{A_I}\right) = \ln\left(\frac{A_R}{A_I}\right)_{t=0} - k'_{app}t_m \tag{11}$$

and

$$k'_{app} \equiv k_m + \frac{f_s k_a k_s}{f_m(k_d + k_s)} \tag{12}$$

On comparison with eqn. 4 for the ideal chromatographic reactor, we see that eqn. 12 indicates how the measurements of the intrinsic reaction rate constant in the stationary phase can be affected by finite adsorption and desorption kinetic processes.

A more general expression for an overall elution profile which contains both reactant and product has been obtained in terms of the first absolute moment [59]. This approach overcomes difficulties in resolving reactant and product overlap in elution profiles and eliminates any need for either internal or external standards.

2.2.4. Case 2. When axial dispersion becomes important and the second-derivative term is significant, the system can be treated again with Laplace transformation and the statistical moment method. The following form can then approximate the solution [58]:

$$\ln\left(\frac{A_R}{A_I}\right) = \ln\left(\frac{A_R}{A_I}\right)_{t=0} + \frac{u_0 L}{2\mathscr{D}_m}\left\{1 - \left[1 + \left(\frac{4\mathscr{D}_m}{u_0 L}\right)\left(k_m t_m + \frac{k_s t_s}{1 + k_s/k_d}\right)\right]^{1/2}\right\} \tag{13}$$

More exact solutions for describing elution profiles are available in the form of statistical moments [73,89] or through numerical methods such as fast Fourier transformations [60]. However, evaluation of actual kinetic and dispersion parameters then becomes more difficult.

From eqn. 13, we can see that the axial dispersion influence on intrinsic kinetic evaluation is insignificant when

$$k_m t_m + \frac{k_s t_s}{1 + k_s/k_d} \ll \frac{u_0 L}{4\mathscr{D}_m} \quad (14)$$

If the column Peclet number ($u_0 L/\mathscr{D}_m$) is much greater than the reaction terms indicated in eqn. 14, one can neglect the effect of axial dispersion on LCR analysis, as eqn. 13 reduces to eqn. 11 after applying a binomial Taylor expansion. For modern liquid chromatography, this criterion is usually satisfactory as the dispersion coefficients of molecules in liquids are small and chemical reaction rates are relatively slow [58,59,93]. However, for situations involving very short columns, low flow-rates or fast reactions, this criterion should be examined carefully. When similar approaches for evaluating mass transfer effects from external film resistance and intraparticle diffusion have been used in the LCR their effects were also found to be insignificant [58,59].

2.2.5. Case 3. Although the linear distribution isotherm assumption is often valid for small pulse injections, it becomes questionable for large sample loadings and with frontal or displacement chromatography [109–111]. Therefore, several types of non-linear isotherms have been proposed for replacing the linear relationship between reactant concentrations in the two phases where LCR models are concerned.

Langmuir isotherms have often been used with gas–solid chromatographic reactors [68,72,74,75,83,84] and sometimes for association behaviors in affinity chromatography [94,112]. They also have been incorporated in studies of the preparative chromatographic reactor [30]. For a multi-component system, they assume the form

$$C_{si} = \frac{\alpha_i C_{mi}}{1 + \sum_j \beta_j C_{mj}} \quad (15)$$

The solutions for LCR models have generally only been accessible with numerical methods. With a limited number of active sites, the Langmuir-type model gave a lower conversion than the linear-type model, especially at high reactant concentrations [30].

Although Langmuir isotherms describe non-linearity satisfactorily in many instances, they are not adequate for a highly heterogeneous surface or when strong interactions between solutes in the fluid phase occur. Because of this, the Freundlich isotherm has been used to treat multi-component, competitive adsorption LCR systems on several occasions [25–27]:

$$C_{si} = c_i C_{mi}^{\gamma_i} \quad (16)$$

In these studies, experimental data for multi-solute adsorption have given good least square fit of the parameters c_i and γ_i, with the reactor models being solved using the finite difference method.

Power law and other empirical equations can also be used to describe non-linear isotherm behavior [75,77]. In general, non-linear effects are less significant in the performance of the chromatographic reactor than chemical kinetics, even for preparative operations [30]. This is especially true when reaction features of the chromatographic reactors are a major focus. On the other hand, when separations or physico-chemical measurements are the main concern, a careful examination of this aspect is appropriate.

The utilization of the principles above both qualitatively and quantitatively in kinetic studies can be illustrated by reviewing their applications in several fields.

3. CHEMICAL KINETIC APPLICATIONS

Modern liquid chromatographic use for post-reaction sample analysis to obtain kinetic information has flourished over the last 10 years. The availability of fast separations with small sample requirements, ease of operation and accurate quantification has greatly facilitated conventional kinetic studies of chemical reactions. Examples of liquid chromatographic applications involving techniques such as spectrophotometric, conductive, electrochemical and radioactivity-monitoring methods for analysis of reaction mixtures to obtain kinetic parameters and mechanistic information abound. These encompass areas of biochemistry, especially pharmaceutical-related enzymatic reactions, and studies of organic and inorganic reactions, including photochemical-type reactions. Nevertheless, this area is still not included in the discussion of liquid chromatographic reactors that we have defined here

as reaction is not occurring inside the column and column use is really for the purpose of separation and analysis.

Although there was some general discussion of reaction and kinetic processes applicable to liquid chromatography by Keller and Giddings in 1960 [46,108], adaptation of the modern liquid chromatograph to chemical reactor use did not occur until the 1970s, and it is only in recent years that experimental applications in this area have witnessed significant progress. Detailed discussion on recent LCR developments is contained in this section. A distinction between two types of liquid chromatographic modes is made here because different reaction patterns and retention mechanisms are involved in each: (3.1) liquid–solid adsorption types and (3.2) bonded-phase types with emphasis on alkyl-bonded reversed-phase chromatography.

TABLE 4

REPRESENTATIVE REACTION KINETIC STUDIES IN LIQUID CHROMATOGRAPHIC REACTORS

Reaction	Mobile phase[a]	Stationary phase	Rate constant[b] (s^{-1})	Ref.
(A) Liquid–solid adsorption chromatography				
Hydrolysis of methyl formate to methanol and acetic acid	HCl–water	Activated charcoal	10^{-3}	25 26, 27
Ethanolic esterification of acetic acid to ethyl acetate	Dioxane–heptane Dioxane–water	Alumina–resin Cation-exchange resin	10^{-2} (70°C)	28 29
Hydroquinone oxidation to benzoquinone	*tert.*-Butanol–hexane	Iron-modified silica	10^{-3}	59, 113
(B) Bonded-phase liquid chromatography				
Base-catalyzed esterification of tetrachloroterephthaloyl chloride and its half-ester	Pyridine (4-picoline) in THF–CH_3OH	ODS	10^{-3}–10^{-4}	16, 17, 98
Papain/lysozyme/STI[c] denaturation	1-PrOH–H_3PO_4–water	C_4	10^{-3}	18, 19
Lysozyme/STI denaturation	1-PrOH–TFA–water	ODS	10^{-2}–10^{-3}	24
Lysozyme conformation	CH_3OH–H_3PO_4–water	C_4	10^{-2} (4°C)	114
α-Lactalbumin unfolding	Ammonium sulfate–water	C_1- and C_2-ether	10^{-2} (4°C)	115
Methoxyhydroquinone oxidation	Acetonitrile–water	C_8	10^{-3}	116
Base-catalyzed hydrolysis of *p*-nitrophenyl esters	NaOH in acetonitrile–water	Polymer-based PRP-1	10^{-2}–10^{-3}	41
(C) Interconversion and fast equilibria				
Cis–trans proline isomerization	Phosphate–water	ODs	10^{-3}	21, 106
RNase A refolding	1-PrOH–H_3PO_4–water	C_4	10^{-2}–10^{-3}	117, 118
RNase A denaturation–renaturation	GuHCl–water	Gel permeation, TSK-G2000SW	10^{-3}	93
Muramyldipeptide anomers	CF_3COOH–CH_3OH–water	ODS	10^{-3} (34°C)	119
Pyranose sugar anomer mutarotation	CH_3COOH–acetone–water	NH_2-silica	10^{-3}	120
Pd(II) dithiocarbamate intramolecular rotation	Isopropyl acetate–hexane	Silica	10^{-3} (0°C)	121
Carbon–nitrogen bond rotation of formanilide and *o*-substituted acetanilide	CH_3COOH–hexane–acetate or 1-PrOH	Silica	10^{-3} (−27.6°C) 10^{-3}–10^{-4} (−40°C)	122 22
Phenyl methylcarbamate intramolecular rotation	CH_3COOH–1-PrOH–hexane	OH	10^{-2}–10^{-3} (−30°C)	23

[a] THF = Tetrahydrofuran; 1-PrOH = *n*-propanol; TFA = trifluoroacetic acid; GuHCl = guanidinium chloride.

[b] First-order or pseudo-first-order kinetic assumption; room-temperature values except when noted otherwise in parentheses.

[c] STI = Soybean trypsin inhibitor.

In Section 3.3, interconversion and fast equilibria studies in liquid chromatographic columns are addressed separately because of the special nature of the reactions involved and the coupling with separation.

In Table 4 some representative reactions studied in modern liquid chromatographic reactors are summarized. Also illustrated in Table 4 is a rule of thumb for choosing a chemical reaction and proper operating conditions for LCR kinetics where rate constants apparently should be in the range between 10^{-2} and 10^{-4} s^{-1}. Reactions with rates below this range can still be studied by using special techniques such as stopped-flow methods. For preparative purposes, studies of reactions with faster rates are also common.

3.1. Liquid–solid (adsorption) chromatographic reactors

Liquid–solid adsorption chromatography received initial attention for reactor application because of a similarity to gas–solid chromatography and the availability of solid adsorbents with well characterized properties. Here, however, isotherm non-linearity and non-ideality due to adsorption kinetics often cannot be neglected. Varying isotherms and possible strong adsorption and slow desorption can be incorporated into models so that moment approaches and numerical procedures are usually required to obtain viable solutions for this type of reactor system.

A pioneering work in this area was the experimental and computational study by Wetherold *et al.* [25] in 1974 on the liquid-phase hydrolysis of methyl formate over an activated charcoal bed to form methanol and formic acid ($A \rightleftharpoons B + C$). A Freundlich isotherm with an empirical correction factor was chosen to treat the multi-component nature of the adsorption system. The possibility of overcoming thermodynamic conversion limitations, *i.e.*, the equilibrium value, was demonstrated. Specifically, because the two products had different affinities for the stationary phase there was sufficient separation to inhibit the reverse reaction. A similar reaction system was selected for study by Cho *et al.* [26,27] in a continuous-flow annular reactor configuration with a rotating feed injection port to achieve the same objective of exceeding the equilibrium conversion. An ideal chromatographic model modified for dispersion effects gave satisfactory agreement with experimental data. A simplified binary-component Freundlich adsorption isotherm was used in the numerical simulation when there was rapid disappearance of methyl formate reactant and no competitive adsorption between methanol and formic acid products.

The ethanolic esterification of acetic acid catalyzed by a cation-exchange resin to give ethyl acetate ($A + B \rightleftharpoons C$) was studied in a liquid–solid chromatographic reactor by Sardin and Villernaux [28]. A mixture of alumina acting as a sorbent and the cation-exchange resin was used for packing material, to separate the desired ethyl acetate product from water and from the reactants which travel at the same speed in a dioxane–heptane mobile phase. The 90% conversion attained far exceeded the 67% equilibrium conversion. Later, a general theoretical treatment of this system involving two shocks [29] was found to agree with experimental data; some deviations from isotherm linearity were discussed.

In addition to the above studies where product yield with reactor performance was the main concern, use of a liquid chromatographic column as a microcatalytic reactor for obtaining kinetic data was demonstrated by Melton *et al.* [123]. The lactase-catalyzed hydrolysis of α-nitrophenyl-β-D-galactopyranoside (ONPG) was used as a model reaction for studying immobilized enzyme activity on porous glass beads. Advantages of adapting a liquid chromatographic system to micro-reactor use for studying kinetics include speed, reproducibility, convenience for non-volatile samples, and the ready adaptation of equipment with little modification. Melton [124] also demonstrated the use of this micro-reactor and a factorial design approach involving nine system variables (see Table 5) to screen immobilized enzyme activity quickly and efficiently. The results from Melton's experiments indicated that the amount of enzyme initially present and the pH of immobilization were the most critical parameters, whereas the Mg^{2+} concentration, the temperature of immobilization and the support mesh size had little effect on immobilization effectiveness under his conditions.

The operation by Melton *et al.* actually resembles and utilizes some principles of the flow-injection analysis (FIA) technique [43,125]. FIA itself can

TABLE 5

VARIABLES IN FACTORIAL DESIGN EXPERIMENTS ON LACTASE-CATALYZED ONPG HYDROLYSIS

Variable No.	Variable	High value (+)	Low value (−)
1	Time of immobilization	90 min.	60 min
2	Sequence of anchoring agent[a] addition	Before enzyme	With enzyme
3	Length of anchor chain to glass bead	C_6	C_3
4	Mg^{2+} concentration	10^{-3} *M*	0
5	Temperature of immobilization	*ca.* 20°C (room temp.)	0°C
6	Amount of enzyme added	20 mg	10 mg
7	Mesh size of support	60–80	80–100
8	pH of immobilization	7	4
9	Anchoring agent[a] concentration	7.5%	2.5%

[a] Glutaraldehyde used as anchoring agent for this experiment.

provide rapid on-line determination of the complexing abilities of individual species. However, for mixtures of complexing species a high-performance liquid chromatographic (HPLC) system is needed [127]. FIA methods based on chemical reactions from the introduction of a continuous stream of reagent(s) and/or the stopped-flow technique are often adapted in post-column reaction detection for liquid chromatography, especially in enzyme kinetic studies. Excellent related studies of immobilized enzyme kinetics took place during the 1970s using HPLC and other types of equipment, some of which are referenced elsewhere [43,123]. However, because these are so well treated in more specialized texts, such as that by Carr and Bowers [181], they are not addressed further here.

Recently, catalytic activities in silica columns and related kinetic processes were examined by Jeng and Langer [113,128]. The oxidation of hydroquinone to form benzoquinone in an organic environment was used as a probe reaction to detect column activities resulting from contamination, probably from transition metal ions leached from the stainless-steel components of HPLC hardware [128]. Whereas flow-rate variation and direction reversal (Fig. 5) were applied to locate active sites in the column, their redox nature was characterized by column treatments with an appropriate oxidant or reductant. Later, the same reaction in a uniform column packed with iron(III)-modified silica was utilized to demonstrate the feasibility of using a statistical moment method based on the overall elution profile of reactant and product to evaluate reaction kinetic parameters [59]. Reactant adsorption kinetics were shown to be important factors in reaction-rate measurements for the low conversion situation occurring at lower temperatures and higher flow-rates. Further, LCR kinetic results were shown to complement retention behavior for the column to characterize the modified surface of the stationary phase [113]. A weaker adsorption effect was observed for the silica sample where strongly adsorbing, hydrogen-bonding surface silanol groups were eliminated during iron treatment. The foregoing group of publications illustrate the variety and kinds

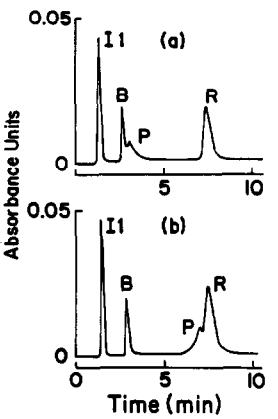

Fig. 5. Effect of direction of flow on liquid reactor chromatogram for 2.5 m*M* hydroquinone (R) oxidation; P = product formed in column, B = benzoquinone in injected sample. (a) Normal flow direction, (b) flow reversed. Column, Altex Ultrasil silica (10-μm); mobile phase, isopropanol–hexane (5:95); flow-rate, 1.76 cm^3/min (150 p.s.i.); sample size, 20 μl; wavelength, 254 nm. The active sites are distributed at the inlet of the column during normal flow direction. (From ref. 128; reproduced from the *Journal of Chromatographic Science* by permission of Preston Publications, a Division of Preston Industries, Inc.)

of information which can be obtained about the packing from diagnostic and kinetic studies where the column is used as a reactor.

3.2. Bonded-phase liquid chromatographic reactors

The stability and variety of available stationary phases and elution modes have been important factors in the growth and popularity of modern bonded-phase liquid chromatography as an analytical technique. Nevertheless, systematic studies using bonded-phase liquid chromatographic columns as chemical reactors did not occur until the 1980s. Some studies of solvolysis reactions of tetrachloroterephthaloyl chloride with alcohol (Fig. 6) by Langer and co-workers in a C_{18} reversed-phase liquid chromatographic reactor were directed toward demonstrating the feasibility of obtaining kinetic information and insights into stationary phase structure and retention mechanisms [16,129]. Specifically, pseudo-first-order rate constants for a base-catalyzed solvolysis were determined in the course of considering reaction in the stationary phase. The base catalysts, pyridine or 4-picoline, were present as homogeneous components in the mobile phase and were thereby also distributed in the stationary phase. The same reaction kinetics were subsequently utilized to attempt to characterize the nature of the chemically bonded stationary phase [17] and to evaluate the phase ratio in a bonded-phase column [130]. (The use of reaction kinetics as a tool for obtaining information on the stationary phase is discussed further in Section 5).

A series of LCR chromatograms from the work above is shown in Fig. 7 to illustrate further advantages of chromatographic reactors. These chromatograms result from the formation of a quaternary ammonium salt (M) initially in the course of the pyridine-catalyzed esterification of tetrachloroterephthaloyl chloride (R). In these chromatograms it can be seen that:

(a) impurities in injected reactant samples show up with characteristic retentions as peaks on the chromatogram and can be accommodated; here, H is the half methyl ester already formed before injection from a slow uncatalyzed reaction (Fig. 6);

(b) side-reactions can be detected on the chromatogram (additional product curves would be perceived [131]);

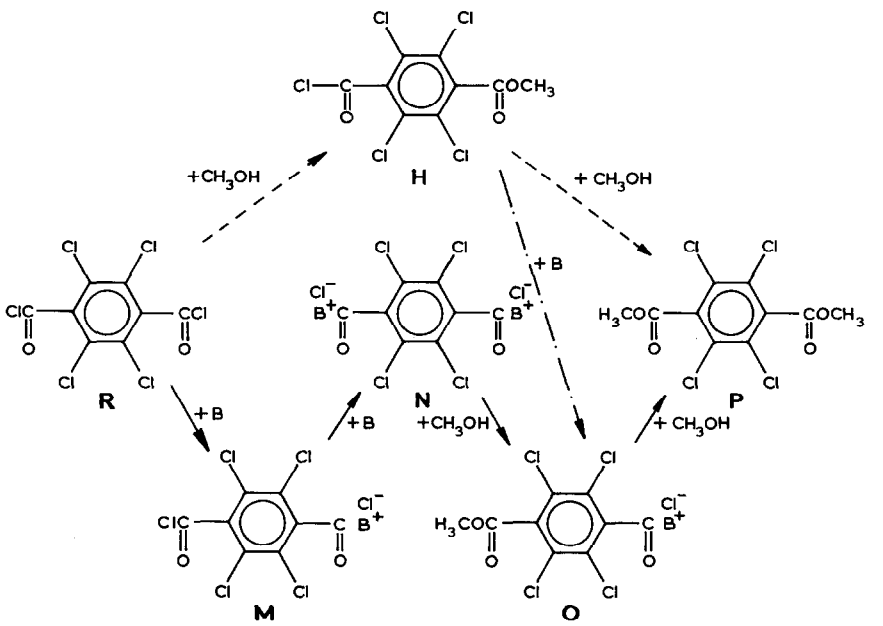

Fig. 6. Reaction sequence (solid arrows) for the base (B)-catalyzed methanolic esterification of tetrachloroterephthaloyl chloride ($TCTPCl_2$), R; uncatalyzed solvolysis sequence in parallel (dashed arrows). B is pyridine or 4-picoline; M, N and O are intermediate quaternary salts; H and P are the half-ester and di-ester products [129].

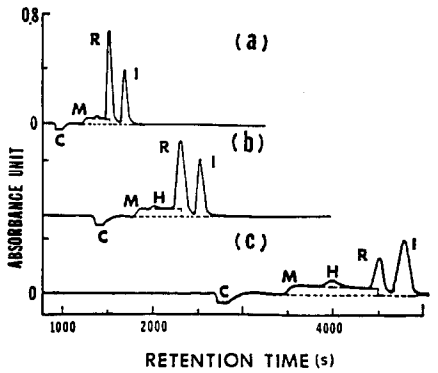

Fig. 7. Series of liquid chromatograms for the $TCTPCl_2$ esterification reaction catalyzed by 0.0075 *M* pyridine–0.123 *M* tetrahydrofuran (THF) in methanol at 35°C. R = reactant ($TCTPCl_2$); M = intermediate product (pyridinium, Cl-TCTP); H = half-ester impurity (methyl, Cl-TCTP); I = inert (1-phenylheptane); C = catalyst vacancy peak (pyridine). Flow-rate: (a) 0.32; (b) 0.21; (c) 0.11 cm^3/min [98]. Reproduced with permission.

(c) even if reverse reaction were to take place later in the column, any change would affect other parts of the chromatogram. With reacted material removed from the original eluting reactant peak, forward kinetic rates can still be ascertained.

As explained earlier, k_{app} can be calculated from eqn. 3 based on information obtained from Fig. 7; then, with a knowledge of k_m, it is possible to evaluate k_s using eqn. 4. Although k_m is usually measured from batch reactor information, a novel reactor configuration recently has been applied to the evaluation of rate constants in both phases simultaneously [98,132]. In this arrangement, a liquid chromatographic reactor was modified to incorporate a void column between two packed columns (Fig. 8). By simply varying the void-column size and the eluent flow-rate, which resulted in simultaneous changes of reactant residence (reaction) times in both mobile and stationary phases, rate constants in each phase could be determined through least-squares fitting of the following equation:

$$\frac{A_p + A_v}{A_I} = C_1 - C_2 \exp\left[-(k_{m,v}t_v + k_{m,c}t_m + k_s t_s)\right] \quad (17)$$

where A_p, A_v and A_I are the peak area of product, void product formation and inert standard, respectively, $k_{m,v}$ and $k_{m,c}$ represent the mobile phase rate constant in the void and packed column sections, t_v is the residence time in the void column and C_1 and C_2 are empirical constants [98,133]. A distinction between $k_{m,v}$ and $k_{m,c}$ can be used to examine the effects of liquid flow patterns on the rate of product formation. Another application of this type of approach to detect any inadvertent void zones and heterogeneities in a chromatographic column has been described [134].

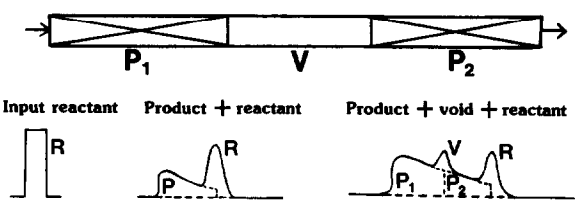

Fig. 8. Illustration of reaction chromatograms in the single void column (P_1–V–P_2) chromatographic reactor array [132]. In the void section, product (V) is formed and remains with reactant. It is separated from the reactant peak and superimposed on the product wave in the next packed column (P_2).

Additional potential for the use of chemically bonded liquid chromatographic reactors for kinetic studies was recognized by other researchers simultaneously with the investigations described above. Some interest arose through the observation of unexpected multiple peaks resulting from on-column reactions during chromatography. Special concerns emerged from studies on interactions between biochemical eluites and packing materials and possible adverse effects. These types of studies have provided *in situ* kinetic information about complex solute–surface interactions, which are otherwise difficult to obtain independently.

The examination of the reactive behaviors of proteins during separation in reversed-phase liquid chromatography by Karger and co-workers is a good example. Kinetics related to the unfolding of papain, soybean trypsin inhibitor (STI) and lysozyme on a hydrophobic *n*-butyl-bonded silica gel surface were studied [18,19]. The denaturation rate was estimated from the measurement of remaining native protein peak area relative to the "incubation" (reaction) time the species resided on the bonded-phase surface (Fig. 9). The suggested mechanistic model for the on-column unfolding process included two steps, one occurring rapidly upon contact of

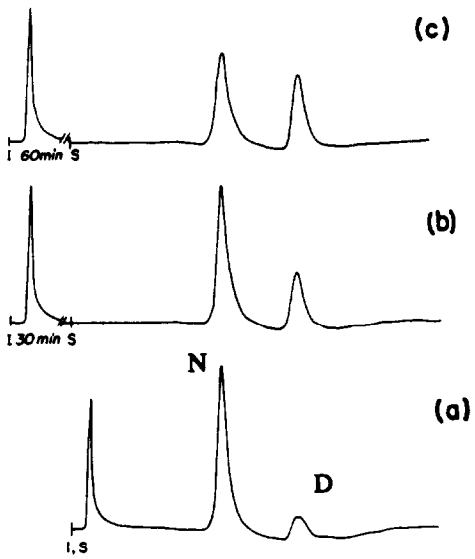

Fig. 9. Chromatographic behavior of papain as a function of on-column incubation time at mobile phase composition of 1-propanol–water (5.4:95.6, v/v) in which the total H_3PO_4 concentration is 10 m*M*. N = native peak; D = denatured papain peak. Conditions: column, C_4 bonded phase on 10-μm LiChrospher SI-500; gradient rate, 3% propanol/min, 15-min linear gradient; flow-rate, 1 ml/min; sample, 20 mg/ml papain, 6 μl injected; detection at 280 nm; column temperature, 5°C. I = Injection time; S = start of gradient. Incubation times: (a) 0; (b) 30; (c) 60 min. (From Ref. 18; reproduced with permission.)

proteins with the stationary phase and the other being a slow denaturation step.

More recently, intrinsic fluorescence has been used with liquid chromatography by Karger's group [114,115] to study the surface dynamics of proteins in contact with hydrophobic silica adsorbents. Grinberg *et al.* [135] have also reported another means beyond conformational changes by which multiple peaks arise. These were a result of protein aggregation during hydrophobic interaction chromatography of β-lactoglobulin A. Hearn *et al.* [136] have illustrated the application of (second) derivative spectroscopy and multi-wavelength detection for monitoring stationary phase-induced and/or mobile phase-mediated effects on protein conformational changes during reversed-phase and size-exclusion liquid chromatographic separation. In these types of kinetic studies, the flow was often interrupted and the eluite immobilized by mobile phase compositional changes to increase the chromatographic dwell ("incubation" or residence time in the column), followed by gradient elution (Fig. 9). Consequently, the reaction proceeded considerably further and the separation of products from reactants was also facilitated. This type of immobilization–gradient flow operation is in principle similar to the "stopped-flow" technique first suggested by Phillips *et al.* [36], which increased the residence time in a localized portion of the column. It is also used in the work of Jaeger and co-workers [40,41] described later.

Another early identification of solute–stationary phase interaction can be found in the study by Huang *et al.* of on-column redox reactions of methoxy-substituted hydroquinones and benzoquinones during reversed-phase chromatography [116]. They found that metal-catalyzed heterogeneous reactions that followed Langmuir–Hinshelwood kinetics occurred at the stationary phase surface. Good evidence that metal ions were acting as active catalytic sites on silica surfaces was also found in the investigation by Cramer *et al.* [137] of complexing reactions of deferoxamine in a similar chromatographic system. The use of probe reactions in liquid chromatographic columns as a diagnostic tool to study the stability of packing materials as well as the medium will probably expand in the future as HPLC is applied more frequently for specialized separations. This concept has been used with columns in connection with adsorption [128], ion-exchange [138] and ion-exclusion [139] chromatography and with preparative columns [140].

3.3. Interconversions and fast equilibria studies

Klinkenberg [64] and Keller and Giddings [46] very early recognized and attempted to analyze chromatographic profiles involving a first-order reversible reaction system where reaction rates are on about the same time scale as the column separation process. A hypothetical chromatogram for a mixture of interconverting species (R $\rightleftharpoons$ P) to simulate the situation is shown in Fig. 10. Here, an interference regime at the depression in the chromatogram where R and P are indistinguishable is the result of some conversion of each of the two species to the other in the course of passage through the column. Separation can be improved where the rate of the overall chromatographic process can be increased relative to the reaction process. This is illustrated in Fig. 11, where a simple *syn–anti*

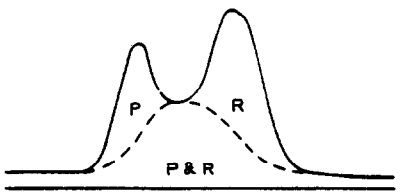

Fig. 10. Hypothetical chromatogram expected for a reversible first-order reaction R⇌P where reaction rates are commensurate with separation processes.

acetaldoxime ($CH_3C=NOH$) interconversion in polyethylene glycol was involved in the work by Yurchak and co-workers [47,141]. The separation of *syn* and *anti* forms was enhanced by effectively lowering the interconversion rate (by decreasing the temperature) and increasing the flow-rate (decreasing the reaction time). By controlling liquid chromatographic operating parameters such as flow-rate, temperature or mobile phase composition and pH with similar considerations, optimum conditions can be identified to achieve improved separations of rapidly interconverting species.

HPLC columns have now been applied as reactors a number of times with increasing understanding to study the kinetics of fast equilibria involving in isomerization, enantiomerization and intramolecular bond rotation [20–24,93,106]. A study by Melander *et al.* [142] reported peak splitting of proline-containing dipeptides in reversed-phase octadecylsilane (ODS) columns. This phenomenon was a result of the slow kinetics of *cis–trans* isomerization which were commensurate with the chromatographic separation process itself. Lowering the pH, increasing the temperature and decreasing the flow-rate all reduced peak splitting as the isomerization rate became fast relative to the separation rate in each instance. These results provide chromatographers and others with useful guidelines for optimizing isomer separations and have stimulated a number of subsequent studies. Lebl and Gut observed a similar plateau region between peaks for α- and β-anomers in the separation of muramyldipeptides in an ODS column [119]. Interconversion rate constants were determined using two experiments; however, these analyses may well have been oversimplified as no distinction was made between reactions occurring in the mobile phase and those occurring in the stationary phase as represented in the following simplified general scheme:

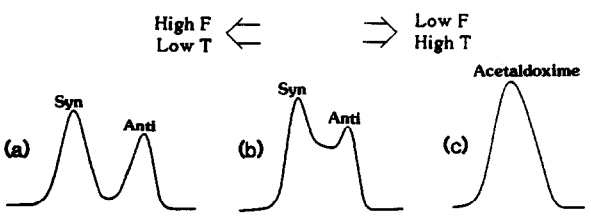

Fig. 11. Reactor chromatograms for reversible interconversion of *syn*- and *anti*-acetaldoxime [141]. T = temperature; F = flow-rate.

$$\begin{array}{ccc} R_m & \underset{k'_m}{\overset{k_m}{\rightleftharpoons}} & P_m \\ \Big\uparrow\Big\downarrow & & \Big\uparrow\Big\downarrow \\ R_s & \underset{k'_s}{\overset{k_s}{\rightleftharpoons}} & P_s \end{array} \qquad (18)$$

Additional studies of *cis–trans* proline isomerization in reversed-phase columns used as chromatographic reactors were reported later by Horváth and co-workers. Dynamic effects resulting from the simultaneous chromatographic movement and first-order reversible reactions in both phases as shown in eqn. 18 were investigated for a linear, ideal case by Melander *et al.* [20]. The dimensionless Damköhler number ($Da = kL/u_0$), representative of the ratio of time constants for bulk mass transport and chemical reaction, was used as a measure of the relative importance of these two competitive processes. The dependence of chromatographic profiles on the Damköhler number showed that the interplay of on-column reaction and chromatographic retention is important only in the range $10^{-3} < Da < 10^3$ in their study. In the limit of *Da* equal to zero, *i.e.*, no interconversion between the two isomers, a complete separation was obtained. At a sufficiently high *Da* value, the interconversion was so fast that no separation occurred and the two isomers eluted as a single merged peak (*e.g.*, see Fig. 11c). Expressions for the first and second moments of the elution profiles were also obtained as a function of *Da*.

Later, the first absolute moment (center of mass) and the second central moment (variance) of the entire concentration profile were measured experimentally during *cis–trans* proline isomerization in an ODS column using a phosphate-buffered aqueous mobile phase [21]. The forward and reverse

interconversion rate constants in the stationary phase could then be calculated. Because the estimated rate constant values in the stationary phase were higher than those in solution, it was suggested that the hydrocarbonaceous-bonded silica surface possessed special catalytic properties for the isomerization. Two recent papers by Hanai and coworkers have discussed an "elution-band relaxation method" to evaluate reversible isomerization kinetics in a linear, ideal liquid chromatographic reactor [93,106]. The theoretical approach was very similar to that of Melander *et al.* [20] and two reactions, denaturation–renaturation of ribonuclease A (RNase A) and *cis–trans* isomerization of proline, were examined experimentally. Plots of the measured first moment for the overall chromatographic profile of the two isomers *versus* inverse flow-rate were fitted with a least-squares analysis to give the "apparent" rate constants for the column.

Interconversion phenomena of the type discussed above have often been observed during separations of biomolecules. For example, Parente and Wetlaufer reported the reversible urea–thermal denaturation of α-chymotrypsinogen-A during ion-exchange chromatography [138]. Karger's group also studied unfolding–refolding effects in reversed-phase liquid chromatography of RNase A where reversible conformational kinetics occurred since the relaxation time for RNase A refolding was comparable to its mobile phase residence time [117,118]. The protein was denatured on adsorption in the bonded phase, and the reversible renaturation took place in the mobile phase in the course of elution down the column. In addition to flow and temperature variations, salt type and mobile phase composition have also been used as operating parameters to optimize chromatographic separations. These and other variables such as stationary phase hydrophobicity [143] and the presence of trace metals [116,137,144] are all important factors to consider when one attempts to understand and control conformational changes of complicated systems of this type during liquid chromatography. Hearn *et al.* have provided an excellent analysis and discussion on general systems involving polypeptides and proteins which undergo conformational changes in both mobile and stationary phases [24]. The observed multi-zoning phenomena can be traced to multi-phasic transitions of proteins during chromatography.

The advantages in understanding and elucidating these types of complicated kinetic effects in the LCR can be appreciated further by reviewing the interesting early work on separations of interconverting isomers at low temperatures in liquid chromatographic columns by Moriyasu and co-workers [120–122,145,146]. As indicated above, a similar approach had been applied in gas chromatography to study the dynamic isomer interconversion process [47,48,81,141]. The classical problem of interconverting dynamics was re-examined with modern liquid chromatography to measure some rates for reactions such as mutarotation [120], keto–enol interconversion [145], and intramolecular bond rotation [22,23,121,122]. In most experiments, the chromatographic systems were operated at unusually low temperatures to slow reactions and overcome the competition from fast interconversion so that the separation of the interconverting isomers could be achieved (*cf.*, Fig. 11). Only later was there an appreciation of complications from the catalytic effects of the acidic modifier (acetic acid) in the mobile phase and acidic groups on some silica packings. With appropriate changes in bonded columns and a more neutral mobile phase, they were able to raise the operating temperatures for isomer separation considerably [146]. Nevertheless, low-temperature LC operation was shown to offer advantages in the study of labile species and fast reactions [147,148]. Although lowering temperature gives some anticipated unfavorable effects on chromatographic efficiency because of slower mass transfer and adsorption kinetics, chemical kinetic effects are still more pronounced because of the relatively higher activation energy of the interconversion reactions.

Related to the above applications are chromatographic studies of secondary chemical equilibria in modern liquid chromatography. Examples of secondary chemical equilibria, in addition to the interconversions discussed above, include acid–base equilibria, ion pairing, complexation and solute–micelle interactions [10]. Liquid chromatography has made it possible to achieve some separations that are otherwise impossible. Several general reviews are available for discussion of physico-chemical aspects of these operations [9,149–151]. In general, few results for kinetic studies of secondary chemical equilibria occurring in liquid columns,

with the exception of interconversions, are available [46,49,149,152]. Hence the potential for applying the LCR to obtaining kinetic data for secondary chemical equilibria and the use of these phenomena with the LCR still await further exploration.

In this section, we illustrated applications of liquid chromatographic reactors to chemical kinetic studies of various types of reactions, such as reversible isomerizations and interconversions and also irreversible solvolyses and surface-catalyzed reactions. Whereas many theoretical solutions for problems in liquid chromatographic reactors have been obtained and some are described in Section 2, fewer experimental applications have been described in the literature. Moreover, assumptions of ideality and/or linearity have usually been used and distinctions between reaction rates in the mobile and stationary phases have sometimes not been addressed. Therefore, more detailed studies of the application of LCR systems to both irreversible and reversible reactions of different complexities are desirable and can be anticipated. Pertinent to this, a brief discussion of complications in LCR systems used for chemical kinetic studies which can originate with other dynamic processes is presented below.

4. KINETIC PROCESSES RELATED TO LCR APPLICATIONS

As indicated earlier, in addition to simple chemical reactions, other types of kinetic processes occurring in columns play important roles in studies of liquid chromatographic reactors. These include axial dispersion, interfacial mass transfer, intraparticle diffusion and sorption–desorption (Fig. 4). Where the rates of these processes are commensurate with chemical reaction rates, LCR systems can no longer be treated as ideal. As these phenomena can be complicated, only a limited number of examples can be examined here to illustrate the approach and the importance of careful analysis of non-ideal LCR systems. Also included in this section is a discussion of association–dissociation phenomena in liquid affinity chromatography. Although no net reaction occurs in most affinity columns, some relevant studies are reviewed below where the results indicate useful implications for non-ideal LCR systems.

4.1. Dynamic processes leading to non-ideal behavior

The development of the Van Deemter equation in 1956 [153] was the foremost, early systematic study of band broadening resulting from dynamic processes such as eddy diffusion, axial molecular diffusion and mass transfer. Subsequently, it has been extensively studied and modified, with consideration of processes such as surface diffusion and adsorption–desorption kinetics to provide better descriptions under various column operating conditions, by the groups of Giddings, Knox, Huber and Purnell, among others [52–54,154]. Recent views of band broadening mechanisms resulting from kinetic processes have been presented by other investigators (*e.g.*, [55,56,155,156]).

Band broadening observed in post-column reaction detectors in liquid chromatography is the result of a combination of contributions from normal spreading processes and chemical reaction. Nondek *et al.* [92,157] described reactor band broadening for a first-order, irreversible reaction. The Gaussian component of band broadening was distinguished from reaction broadening so that the results produced a quantitative relationship between the rate constant and the variance from reaction broadening. The influences of flow velocity, mobile phase composition and reaction temperature on band broadening from reaction were also examined to determine the optimum reactor operating conditions using carbamate hydrolysis catalyzed by a basic ion exchanger as a model reaction [157]. Shih and Carr also discussed a strategy for minimization of band broadening in post-column reactors which resulted from the presence of both dispersion and chemical reaction [158]. An approach to flow-rate and reactor size optimization was described to obtain sensitive reactor–detector performance.

Marshall *et al.* studied the contribution of sorption–desorption kinetics to band broadening in an octadecylsilica ion-pairing liquid chromatographic system [159]. The dynamics of sorption–desorption on several modified silica surfaces in liquid suspension were measured with experiments outside the column using a pressure-jump relaxation method. The results indicated that this process plays a significant role in the stationary phase performance. Higher column efficiencies were usually found with columns containing materials where relatively rapid sorption–desorption equilibration processes occur-

red. More recently, Lin and Ma studied the effects of pore diffusion and adsorption with both microporous and macroporous adsorbents in liquid chromatographic columns [160]. Adsorption importance relative to pore diffusion was found to be a function of adsorbent pore size. Their results indicated that both processes were important in describing macroporous adsorbents such as activated alumina, while a pore diffusion model was adequate for microporous silicalite adsorbents.

Conclusions from the studies described above should be applied with care to chromatographic reactor systems as the experiments were performed under conditions where no chemical reaction was occurring. Of course, studies of mass transfer and kinetic effects in the LCR have been limited since *in situ* estimations of transport and kinetic parameters become more elusive when these measurements are coupled with chemical reactions. Suzuki and Smith first examined moment solutions for a first-order surface reaction occurring in a chromatographic system in the presence of some non-ideal processes [50,73]. Wetherold *et al.* [25] and Cho *et al.* [26,27] modified the ideal reactor model by simulating axial dispersion effects to improve descriptions of experimental elution curves.

Recently, mass transfer effects on reaction rate evaluations in liquid chromatographic reactors were systematically examined in our group [58]; included were longitudinal diffusion, intraparticle diffusion and interfacial sorption–desorption. On applying some criteria based on dimensional analysis to recognize the onset of such effects, it was concluded that none of these processes significantly affected reaction kinetic measurements in the $TCTPCl_2$ solvolysis reaction study mentioned earlier. These criteria were used again with the iron-catalyzed hydroquinone oxidation in silica columns with similar conclusions, except that it was found that adsorption and desorption processes could exert some influence on the evaluation of intrinsic kinetics because of the adsorptive nature of the stationary silica bed [59]. A better fit of the kinetic data was obtained when the ideal reactor model was altered to incorporate consideration of finite adsorption kinetics to describe the LCR system. The approaches described here and other more advanced efforts to examine the significance of non-ideal processes relative to chemical reaction are likely to contribute to the future understanding and identification of governing dynamics in chromatographic reactor systems.

4.2. Association–dissociation kinetics and affinity chromatography

Miyawaki *et al.* [161] have described an interesting variation in the application of the chromatographic reactor concept with affinity chromatography. In contrast to most chromatographic reactor applications where reactants or products are adsorbed in the column, the design of their affinity chromatographic reactor took advantage of the strong retention of a cofactor which is a part of the biocatalyst system. Pyruvate and ethanol substrates for lactate production were supplied continuously into a hollow-fiber capillary reactor with an immobilized enzyme system of alcohol and lactate dehydrogenases, while the necessary cofactor, NAD, was supplied as a periodic pulse. The strong affinity of the cofactor for the immobilized enzyme led to NAD pulse retention times which were considerably longer than those of substrates or products. The consequence was very high cofactor turnover numbers. Further, the pulsing cofactor mode facilitated the recovery of expensive NAD, avoiding the instability and control problems of an alternative method where chemically modified NAD was bound and co-immobilized with the enzymes in a membrane reactor.

Association and dissociation dynamics involving solutes and ligands in affinity chromatography also might be considered illustrative of chemical kinetics occurring in the column. While such kinetics are pertinent, it can be noted again that they do not generally result in a net chemical change for the solute in the course of column passage. In 1975, Denizot and Delaage [88] developed a statistical treatment of affinity chromatography, based on the random walk model of Giddings and Eyring [107], to determine the rate constants for association and dissociation reactions involved in separations. Later, Weiss discussed the limitations of this approach for multiple binding sites [162]. In 1978, Horváth and Lin [56] developed a plate-height method for evaluating the association and dissociation kinetics for eluite molecules and the stationary phase in liquid chromatography. Hethcote and co-workers also presented a complete statistical

moment model for affinity chromatography which incorporated both physical and association kinetics [91,163,164]. These fundamental studies have accelerated applications of modern affinity chromatography to kinetic studies. One example is zone-interference chromatography based on the application of stochastic theory to the behavior of solute molecules to determine macromolecular kinetic constants [165].

Muller and Carr applied the theory developed by Horváth and Lin to study some thermodynamic and kinetic characteristics of the interaction between sugar eluites and silica-bound concanavalin A (Con A), a biospecific adsorbent in high-performance liquid affinity chromatography [166,167]. They showed that the observed large plate heights mainly resulted from the sluggish chemical kinetics of the Con A–sugar interaction rather than other mass transfer terms. It was also found that at high sugar concentrations the predominant effect on plate height was isotherm non-linearity [167]; however, no satisfactory quantitative plate theory was available to treat this effect. Subsequently, Anderson and Walters, studying a similar system [168], proposed that diffusion contributions could be larger than indicated by Muller and Carr. Moore and Walters also described a peak-decay method to determine the dissociation rate constant from the slope of the logarithm of the peak tailing response in affinity chromatography [169]. This approach was verified experimentally in the Con A–sugar system with addition of a competitive absorbate (mannose) to prevent re-association.

An interesting "split peak" phenomenon often observed in affinity chromatography has also been utilized in kinetic studies. First predicted by Giddings and Eyring in 1955 [107], the split-peak behavior of a single solute is characterized by a non-retaining peak and a strongly retained peak. This usually undesirable artifact is caused by slow adsorption kinetics or mass transfer of large molecules, and should not be confused with the peak splitting observed in the separation of interconverting species discussed earlier. The phenomenon was used to advantage in the evaluation of rate constants for the antibody–antigen complex formation reaction by Sportsman *et al.* [170]. Later, the theory was expanded by Walters and co-workers to systems where either slow diffusion or adsorption was rate-limiting [171,172]. Non-linear elution effects on measurements of diffusional and adsorption parameters resulting from large sample loads in split-peak chromatography also have been discussed recently [173,174].

Effects of non-linear isotherms on affinity chromatography performance have also received the attention. Chase used a non-linear, sorption rate-limited model to predict breakthrough curves in frontal analysis during preparative affinity chromatography and was able to measure binding kinetics for some biological macromolecular systems [112]. Arnold and Blanch developed a non-linear theory that allowed the effect of mass transfer to be separated from binding kinetics in both zonal and frontal affinity chromatography [94]. Later, Wade *et al.* [175] developed an impulse input method for making physico-chemical measurements in non-linear systems with some advantages over the above methods and discussed implications for the design of preparative-scale operations. With increasing interest in using affinity chromatography for preparative purposes, non-linear effects are likely to receive still more attention in the future.

5. SPECIAL APPROACHES AND APPLICATIONS

Liquid chromatographic features, including the capacity for handling heat-sensitive and non-volatile compounds which led to its widespread application, also make the development and understanding of liquid chromatographic reactors highly attractive. A number of kinetic applications of liquid chromatographic reactors and some of the complexity have already been addressed above. Thus, we see that direct and some indirect information on chemical reactions as well as other associated rate processes can frequently be extracted from reaction chromatograms.

Perhaps the most important conclusion from the work above is that the liquid chromatographic reactor is useful and operable but requires more extensive scrutiny and treatment in many instances than might be obvious at first glance. While implementations for chemical kinetic studies has been proven feasible, LCR systems also can be applied with advantage to other aspects of column operations such as characterizing the stationary phase bed or improving reactivity and/or selectivity for reac-

tions occurring in columns. LCR-type operations might well be feasible now for some preparative purposes. Some of these possibilities are developed further below to demonstrate the potential information available from LCR operations.

5.1. Characterization of column beds

Earlier, we alluded to the use of a chemical tracer method by Deans and co-workers [34,35] to determine residual oil saturation in reservoirs. The approach resembles the use of a liquid chromatographic reactor for characterizing a stationary bed, with flowing water funtioning as a mobile phase and the oil bed in porous strata serving as the stationary phase. Reactant tracer, ethyl acetate, was introduced as pulses into wells where unknown amounts of residual oils existed in the porous rock structure. The well was then sealed to allow some portion of the tracer to hydrolyze to form ethanol, the product reference material. While ethyl acetate is soluble in both water and oil phases, ethanol is most soluble in the water phase. Thus, on reversal of flow in the oil bed, the ethanol reached the well head first as it was retained significantly less within the oil phase than the unreacted ethyl acetate tracer. The difference in arrival times to the well head could then be correlated with residence in the oil phase and serve to measure the extent of residual oil saturation in the rock.

In Section 3, we introduced a few examples of surface-catalyzed liquid chromatographic reactions. The work of Karger, Horváth and others [18–24,116,137] has indicated further possibilities for stationary phase characterization and column design. In much of this work, increased reaction rates are a result of stationary phase surface activities. From the types and extent of reactions involved, one can characterize the catalytic nature of the column beds, *e.g.*, surface hydrophobicity [18,19,21,24,143], hydrogen bonding or acidic groups on silica surfaces [22,23,145,146], and metal ions associated with packing material or leached from the stainless-steel components of an HPLC system [116,128,137,144]. On the other hand, when reactions can occur in both homogeneously mobile and stationary phases, there are possibilities for determining the stationary phase composition through interpretation of kinetic data, as shown by Chu, Langer and co-workers in the course of characterizing a chemically bonded stationary phase with LCR reaction kinetics [16,17,130,176]. Thus, the kinetic data become tools for obtaining further information on the reaction environment rather than an end in themselves.

The statements above can probably best be understood by turning to an interpretation of reactor chromatograms with an octadecylsilyl phase (ODS, C_{18}) such as those in Fig. 7 together with eqn. 3. Our earlier study of $TCTPCl_2$ kinetics showed that the presence of methanol was required for initial reaction to form the quaternary salt intermediate, M [129]. Using the assumption that reaction occurred only in the methanolic mobile phase, calculated on-column solvolysis rates were found to be *ca.* 30% higher than those measured in bulk methanol [16]. The discrepancy could be explained by taking into account the possibility that reaction takes place in the stationary phase also with some associated methanol playing a role. A stationary phase rate constant, k_s, can be calculated from eqn. 4; this value is then compared with those estimated on the basis of two different bonded stationary phase models involving methanol and hydrocarbon ligands to gain information about the chemically derivatized phase.

With the methanol mobile phases employed in our work, one can consider two scenarios [17]: (1) solute molecules interact with an associated methanol "pseudo-layer" without the direct participation of the underlying hydrocarbon ligands [4] (model I); and (2) solute molecules interact with methanolic sheathed bonded hydrocarbon ligands, the sheaths being held by a dispersion type of interaction [6] (model II). The situation is broadly represented in Fig. 12. A stationary phase rate constant value can be calculated for each model for comparison with that obtained from experiments. The observed rates based on the chromatographic reactor results generally tended towards model II, although not completely. One can conclude that the "pseudo-phase" was of a composite type with contributions from both models I and II [17,176]. The approach outlined above, then, is illustrative of how reaction kinetic data can supplement spectroscopic and retention studies and contribute to elucidating the nature of the stationary phase atmosphere, and thus proposed retention mechanisms, in chemically bonded chromatographic systems.

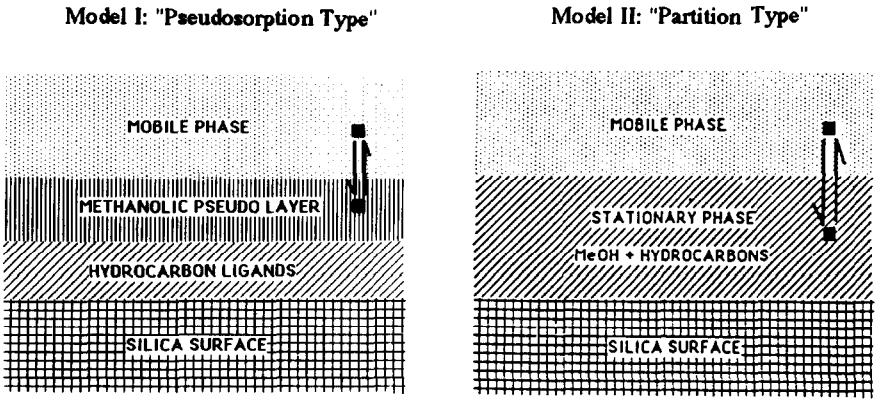

Fig. 12. Models for molecular retention in reversed-phase liquid chromatography with methanol solvent and hydrocarbon ligands. ■ = Solute molecules [176].

Related to the above study is a determination of the phase ratio in bonded-phase liquid chromatographic systems. This determination is elusive owing to the nature of the composite chemically derivatized stationary phase, modelled above, and problems in interpreting solute retention behavior. The phase ratio (φ) for a column is defined as V_s/V_m, where V_s and V_m are the stationary phase and mobile phase volumes, respectively. Because the boundary between these two phases in bonded-type chromatography is often ill-defined and dependent on mobile phase composition, phase ratio determinations have been limited. Continuing the above study, an equation was derived to obtain a value for the phase ratio of the chemically bonded ODS system on the basis of measured LCR kinetics [130]. Thus,

$$\varphi = \sqrt{\frac{k_c' \; k_m}{k_s} \; \frac{V_{s,CH_3OH}}{V_m}} \tag{19}$$

where k_c' is the catalyst capacity factor and V_{s,CH_3OH} is the volume of associated methanol pseudo-layer which is determined from an adsorption isotherm experiment. With known kinetic data for both phases, the phase ratio can be determined. Then, solute retention can be treated on a quantitative basis in the chemically bonded chromatographic system.

The approach utilizing LCR kinetics as described above is an operational one. The calculated phase-ratio values were fairly consistent for different catalyst solutes (pyridine or picoline). Although this result might seem satisfying, it is desirable to carry out additional measurements using other reaction kinetics. Such agreement is required if this method is to be utilized in the future with confidence. Most important here, however, is noting how simple LCR kinetic experiments can provide a basis for interpreting stationary phase characteristics.

5.2. Modifications of reactivity and selectivity

The potential for using chromatographic reactors to enhance both production and selectivity during chemical reactions has created further interest. In principle, the operation is advantageous only for certain reaction systems. In the case of reversible reactions such as $A \rightleftharpoons B + C$ or $A + E \rightleftharpoons R + S$, the reactant conversion can be driven well beyond equilibrium conversion limits of conventional reactors because the possibilities for reverse reactions are minimized through product separation or removal. Some applications have been reviewed by Villermaux [51]. Examples pertaining to liquid chromatographic reactors [25–29] already have been discussed.

Chromatographic reactor operation can also be employed advantageously for reactant conversions where consecutive reactions are involved. For instance, if we consider

$$R \xrightarrow{+B,k_1} M \xrightarrow{+B,k_2} N \tag{20}$$

the goal might well be to improve the yield of intermediate species, M. Indeed, it has been shown that the selectivity and yield of intermediate products for consecutive reactions can be improved in

pulse or moving-bed chromatographic reactors compared with fixed-bed reactors [79,95,177]. Further, intermediates can be recovered with enhanced purity because of the chromatographic separation features involved in intermediate removal with retardation of reactant in the column. One example is in the sequence of reactions for catalyzed $TCTPCl_2$ solvolysis shown in Fig. 6; here, the early elution of the first intermediate, pyridinium, Cl-TCTP (M), eliminates the possibility of further reaction to other products in the column (Fig. 7). On the other hand, the pulsed chromatographic reactor may not be adequate for separating M from B effectively to give yield improvement unless R and B are eluted at the same time [30,51,95].

For other irreversible reactions operating in chromatographic reactors, the separation features above normally give no advantage for achieving higher production. For slow reactions, enhancement of conversion can be achieved through increase of reactant contact time with catalysts in the reactor bed. This effectively lengthens the column to provide increased volume for reactions. An example of utilizing this concept in LCR operation is the affinity chromatographic reactor of Miyawaki *et al.* [161] reviewed earlier, where the high catalyst turnover was a result of relatively selective retention of the cofactor on the immobilized enzyme. The stopped-flow technique adapted for measurements of slow kinetics as mentioned earlier also has potential for enhancing reaction productivity, especially in the LCR because sample dispersion during the stopped-flow interval is minimal in liquids [178]. For example, a chromatographic stopped-flow method combined with varying eluent strength was used to overcome the slow dissociation kinetics of the sugar–Con A complex [38]. This resulted in a larger peak area (*i.e.*, fractional recovery) and diminished peak tailing.

Beyond the utilization of some special concerted separation and reaction features, reactivity and/or selectivity in chromatographic reactors can be enhanced through modification of stationary phase properties. Whereas LCR applications to heterogeneous catalysis are conceptually simple and have ample precedent, the utilization of interfacial hydrophobic and hydrophilic properties to enhance the reactivity and selectivity of liquid chromatographic reactions is more complex and less familiar at present. For slow biphase reactions involving miscibility problems between organic substrates and water-soluble reagents, there is the possibility of altering reactivity and selectivity in designed chromatographic reactors through utilization of the principles of phase-transfer catalysis or the action of surface-active agents. This can be illustrated by the work of Tanaka *et al.* [179] and Jaeger and co-workers [40,41] described below on reactions which are interfacial in nature and involve both organic and aqueous species.

The utilization of interfacial properties in HPLC was first directed toward strengthening its separation potential, as with most other aspects of HPLC development. One prominent example involved the application of micellar liquid chromatography [10]. The potential for utilizing associated principles for enhancing reactivity and/or selectivity of reactions in HPLC columns was described first by Tanaka *et al.* in 1984 [179]. Two nucleophilic displacement reactions were studied at phase boundaries between an aqueous nucleophile (sodium iodide or sodium acetate) and an alkyl halide (*n*-octyl bromide or benzyl chloride) associated with alkylsilylated silica surfaces through hydrophobic interactions. Higher reaction rates and improved selectivity for product formation resulted from using immobilized organic phases in the column, as compared with reacting the halides in a conventional liquid–liquid biphase system. The results suggested that the nucleophilic displacement reactions involved took place at the interface.

Other selectivity studies of liquid chromatographic reactions with polymer-based reversed-phase columns have been reported by Jaeger and co-workers on several occasions. For reactions of compounds with similar intrinsic reactivity (in solution) but different relative hydrophilic/lipophilic character, reaction selectivity could be altered using interrupted flow procedures involving adsorption followed by selective reaction on desorption. For chlorination of *n*-alkyl phenyl ethers, for example, the ether reactant was first introduced and immobilized on a C_{18} column; on introduction of a chlorine–water mobile phase followed by acetonitrile–water gradient elution, selective chlorination favoring *para* over *ortho* positions was observed [40]. The organic-bonded phase and/or the bonded phase–aqueous mobile phase interface were hypothesized to be the

reactive environment and critical to determining the observed selectivity. Another variation of this approach involving an ionic, inorganic reagent reacting with competitive organic substrates was demonstrated in a study of base-catalyzed hydrolyses of *p*-nitrophenyl esters [41]. In the procedure, acetate and hexanoate esters were adsorbed on polystyrene–divinylbenzene-type packings. This was followed by aqueous base, mobile phase treatment and acetonitrile gradient elution. Saponification of the acetate ester was favored relative to the hexanoate, presumably because of the order of desorption of these materials. This type of selectivity was less pronounced at lower ratios of acetonitrile to water where retention differences between the two ester substrates were less pronounced. From the above discussion, it can be seen that there are opportunities for the development of selective reaction schemes here for the small-scale preparation of valuable materials and for reaction of compounds present in trace amounts in the mobile phase.

5.3. Large-scale application

The actual use of an LCR on a production scale has been addressed only recently and is still in an early stage of development. Where an aqueous mobile phase can be employed some procedures already look attractive. Examples from earlier papers [25–30] addressed situations where enhanced product yield for reversible reactions was a major concern. As explained earlier, applications of the LCR are also compatible with a strategy for improving the yields of intermediates where sequential reactions are involved through selective elution. The potential for reduction of downstream purification costs where the LCR is applied is another positive aspect where economics are important. With these features, the chromatographic reactor becomes a prominent candidate for consideration when significant production of costly chemicals involves either reversible or consecutive reactions [51]. For example, Schweich and Villermaux carried out a feasibility study of preparative chromatographic reactors in 1982 [30]. In evaluating their potential, they concluded that broad injections and poor column efficiency were tolerable and that chemical kinetic rate limitations were probably the most important hindrance to efficient chromatographic reactor utilization. Further, they emphasized that extent of reaction with the separation feature, beyond mere conversion alone, is important for an LCR operation to become attractive. A major trend in adapting chromatographic reactor strategies has involved studies of possibilities for using rotating annular beds or moving beds to provide what is effectively continuous chromatographic reactor operations [26,27,74,83,84].

Only a few LCR-type preparative applications of practical industrial interest have been seriously considered. One is a process for producing a high-fructose syrup from glucose reported by Hashimoto *et al.* [31]. A series of stationary immobilized glucose isomerase reactors were arranged alternately with Y zeolite adsorption columns in an array where the inlets were continuously advanced. As all other inlets and outlets were fixed, the columns selectively adsorbed fructose to simulate continuous countercurrent contact of the liquid stream with the stationary bed without actual movement of the solid adsorbent. This process tends to consume less desorbing material than processes involving a separate reactor coupled with either fixed-bed or moving-bed adsorbers, while producing an aqueous syrup with a 45–65% fructose content.

The biosynthesis of a dextran polymer of higher molecular weight from sucrose has been studied recently in a chromatographic reactor by Zafar and Barker [32]. The byproduct, fructose, was retarded through complexation with the calcium ions of the immobilized resin, while the desired dextran product and the sucrose substrate were size excluded and eluted in order. Owing to the substantial denaturation rates of the dextransucrase catalyst, it was necessary to add enzyme continuously to the mobile phase, thus increasing production costs. The separation of the reaction mixture components, however, can result in downstream product purification cost savings while facilitating reactant recycling. Recently, Barker and Ganetsos prepared a stimulating review describing some principles and identifying some potential applications of the LCR in biotechnology [33].

As non-linear and non-ideal processes may not be important in obtaining analytical-scale data, such data cannot be used with confidence for predicting the performance of a preparative chromatographic reactor during scale-up [109–111,178]. Experience together with the elucidation and understanding of

important features of liquid chromatographic reactor operation as suggested here should help to solve the problem in many instances.

6. CONCLUSIONS

Starting with some well defined concepts and the ideal chromatographic reactor, we have seen how operational and equipment modifications can be made to encompass a range of applications, approaches and interfacial situations. Because of this range, only a few illustrative examples of the utilization of the liquid chromatographic reactor could be presented in each area. Earlier extensive gas chromatographic reactor studies helped accelerate applications of liquid chromatographic reactors. However, more complicated processes in the liquid system have often caused some difficulty when one wants to characterize reactor behavior. Further, there has been some compartmentalization of research in several areas so that related techniques have tended to be developed independently (*e.g.*, "stopped flow" and "surface incubation"). Therefore, knowledge of liquid chromatographic reactors compared with gas chromatographic reactors is less advanced at this time, so that further application and understanding emerge as a special challenge for the future. Interesting and attractive possibilities exist. We hope that this review will foster the more widespread use of the LCR in simple situations and improved integration of efforts from multifold investigations in more complex situations.

One unexploited area so far has been in large-scale LCR applications to the treatment and perhaps removal of undesirable materials. This could be from bulk chemicals, physiological fluids or the environment as a whole [41,45,180]. Up to now, the major contribution of chromatography to these types of problems has been as an analytical tool for the identification of undesired or dangerous species. Standard chemical treatments involving fixed-bed adsorbers and reactors have had only limited use for the removal or destruction of toxic materials where complete conversions or selectivity are desired. Alternative incineration techniques are expensive, sometimes difficult to operate and questionable for removing low concentrations of material from fluid wastes. In many situations, concentration of wastes is necessary before treatment and the separation of small amounts of materials from bulk non-toxic fluids can be difficult with high costs. Chromatographic reactors might be applied in a variety of ways to solve these problems. Providing the advantages of higher (sometimes 100%) product or intermediate yields in reversible or consecutive reactions and possibly lower separation costs with the development of large-scale operation, liquid chromatographic reactors can become an additional tool available to environmental engineers. This is particularly true where only a small amount of material must be removed from large amounts of bulk fluid or only limited chemical transformation is needed to detoxify or start the detoxification process.

Taking a wider view, we can recognize that we are dealing with the elucidation of broad, varied situations involving concerted reactions with separation during two-phase flow. This can cover many types of operations that range in scale and type from flow through capillary veins to subterranean terrestrial beds. The treatment of the phase boundary and surface variations and defining interfacial structure are problems in these areas as they are with liquid chromatographic reactors. Sometimes these or combinations of conditions can be altered to advantage to attain more insight or a particular conversion. Further research and advances with an integration of knowledge among investigators should be forthcoming and can be expected to be beneficial to progress in many areas.

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