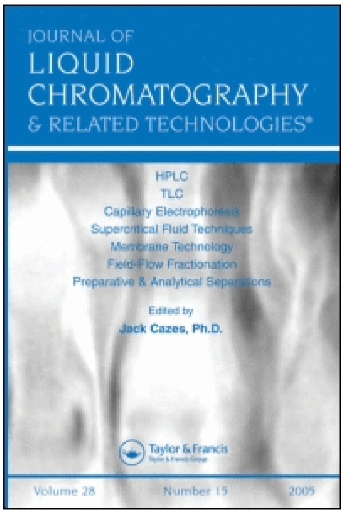

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COMPARISON OF DYNAMIC BEHAVIOR OF C18 HPLC COLUMNS BY STIMULUS-RESPONSE ANALYSIS. I. DETERMINATION OF PECLET NUMBERS

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ABSTRACT

In this study, the dynamic behaviour of C18 HPLC columns is determined by using the stimulus-response technique. Acetonitrile was used as a non-interacting tracer through the C18 HPLC columns (MikroPak® and ELSIsphere®). The system was pulse stimulated with 10 μl of acetonitrile at the flow rates of 1, 2, 3 and 4 ml/min of HPLC grade water. The resulting chromatograms so called response "C" curve is obtained for both columns, separately. The kinetics of that tracer were evaluated in a model study. The model equation was solved using parameter estimation by cybernetic moment technique" and than Peclet numbers, which is the basic indication of the dynamic flow characteristics of the columns, and axial dispersion coefficients, were calculated.

INTRODUCTION

High performance separation techniques like high performance liquid chromatography (HPLC) first gained acceptance mainly for the analysis of high boiling, thermolabile or ionic compounds. Due to the enormous available instrumental diversity, it is possible to adapt the instrumentation of separation parameters almost optimally to the relevant problem. Separations in HPLC systems are based on the interactions of the solute both mobile and stationary phases. However, by changing the column, it is possible to perform many different separation mechanism including normal phase, reverse phase (RP), ion-pair, and ion-suppression chromatography [1]. Among them reverse phase chromatography (RPC) is the most widely used HPLC technique for the determination of a wide variety of substances that have a great importance in food analyses such as sugars [2], vitamins [3], organic acids [4], anthocyanins [5], carotenoids [6], food colors [7], mycotoxines [8], herbicides [9] and pesticides [10].

RP columns are prepared by reacting the surface silanol groups (Si-OH) of support particles with various reagents such as trimethylsilyl (C1), butylsilyl (C4), octylsilyl (C8), and octadecylsilyl (C18) groups [1]. Columns used in RPC have different characteristics affecting the degree of separation of a compound to be analyzed. Parameters controlling separation in a RP column are column dimensions (length, diameter), type and particle size of column packing material and ratio of carbon loading. End-capping which directly affects retention time of a solute during elution is also an important property of RP columns.

In most of researches carried out by HPLC, only column dimensions and type and the particle size of packing material are used to be indicated. However, it is not possible to explain the reasons of changing results obtained for two different columns having similar properties by using same mobile phase compositions at the same conditions.

Stimulus-response methods are well developed in chemical engineering processes for measuring rate and equilibrium parameters such as mass transfer coefficients, diffusivities and adsorption rate constants [11-13]. The moments of the response curves to pulse inputs have been extensively used in the analysis of packed bed systems [14-17]. The first biological application of this technique was made by us [18-20]. In the present study, a modified bead-column test using stimulus-response approach was employed to investigate the dynamic interaction between the mobile phase, HPLC grade water and the packing material C18. Reverse phase columns packed with C18 beads were subjected to a series of tests by stimulus-response technique utilising actonitrile as a non-interacting tracer with the packing material. The column was pulse stimulated and the response of the column was measured.

In this study, flow characteristics of two different RP C18 colums MicroPak® and ELSIsphere® were investigated by stimulus-response analysis and the response curves were evaluated with moment technique, then the results were reported in terms of Peclet numbers which indicates the dynamic behaviour of the column under different flow conditions.

THEORY

The model equation for a packed column with axial dispersion is [20, 21].

$$D \frac{\delta^2 c}{\delta x^2} - U \frac{\delta c}{\delta x} - \rho_p N_A' = \varepsilon \frac{\delta c}{\delta t} \tag{1}$$

where N_A' is the net flux to surface (mol/g.s).

The boundary and initial conditions are based upon Danckwerts' boundary conditions [21], so that for a Dirac delta function pulse input;

Boundary condition I. at $\xi=0$; $M=J(s)_{\xi=0} - \left[\frac{1}{Pe}\frac{\delta J(s)}{\delta \xi}\right]_{\xi=0}$ (2)

Boundary condition II. at $\xi=1$; $\frac{\delta J(s)}{\delta \xi} = 0$ (3)

where;

M: Amount of tracer injected.

For the inert tracer, substituting

$$N_A' = 0 \tag{4}$$

Eq. (1) was written in the Laplace domain as

$$\frac{1}{Pe}\frac{\delta^2 J}{\delta \xi^2} - \frac{\delta J}{\delta \xi} - \frac{\varepsilon}{U}\frac{L}{}\frac{\delta J}{\delta t} = 0 \tag{5}$$

Equations (1) to (3) can be solved in the Laplace domain for J=f(x,s). Then using the relation

$$m_n = -1^n \lim(s \Rightarrow 0) \frac{d^n \overline{J}_A}{ds^n} \tag{6}$$

theoretical moment expressions were derived. For the zeroth moment and first absolute moment and second central moment for the column itself, the result is

$$m_0 = M \tag{7}$$

$$M_1 = \frac{m_1}{m_0} = \frac{\varepsilon L}{U} \tag{8}$$

and

$$M_2^{\#} = \frac{m_2}{m_0} - M_1^2 = \frac{2}{Pe} - \frac{2}{Pe^2}[\,1 - e^{-Pe}\,] \tag{9}$$

Experimental values of the zeroth, first and second moments can be determined from the observed response peaks using the following equations [22].

$$m_0^E = \int_0^a C dt \approx \sum_{i=1}^{n} c_{av.i} \Delta t \tag{10}$$

$$m_1^E = \frac{\frac{1}{2}\sum_{i=1}^{n} c_{av.i}(\tau_{i+1}+\tau_i)}{\sum_{i=1}^{n} c_{av.i}} \tag{11}$$

$$m_2^E = \frac{\frac{1}{3}\sum_{i=1}^{n} c_{av.i}\left[\tau_{i+1}^2+\tau_{i+1}\tau_i+\tau_i^2\right]}{\sum_{i=1}^{n} c_{av.i}} \tag{12}$$

EXPERIMENTAL

High-performance liquid chromatograph: Varian Star model liquid chromatograph was used. It was equipped with a Rheodyne model 7161 six-way injection valve, 10 μl loop, and a Varian model 9050 variable wavelength UV-VIS detector set at 276 nm and 0.02 AUFS. Varian model 4400 integrator was used with a chart speed of 4 cm/min to record resulting chromatograms.

Columns: Two columns supplied from MicroPak® and ELSIsphere®, made of stainless steel, 150 x 4 mm (id), packed with C18 octadecyl groups (5 μ), operated at ambient temperature, were used.

Mobile phase: Bi-distilled water, filtered through a regenerated cellulose acetate membrane (0.45 μ) and degassed ultrasonically was used as the mobile phase with flow rates of 1, 2, 3, and 4 ml/min.

Tracer: Acetonitrile (Merck) was selected as non-interacting tracer to determine the reference response of the columns.

Stimulus-Response Analysis: The system "pulse" stimulated by adding 10 μl of acetonitrile as inert tracer to water eluent at flow rates of 1, 2, 3, and 4 ml/min to determine the reference response of the columns. The column response, which is the so-called "C curve", was determined by following the absorption of the acetonitrile in the eluent stream. In each case the absorption was detected by a UV-VIS spectrophotometer. All the experiments were carried out at the temperature of 25 ^{0}C. The details of the experimental procedure was given elsewhere [23].

RESULTS AND DISCUSSION

Figure 1. and Figure 2. exemplifies the response "C" curves obtained in the stimulus-response experiments in the case of no-adsorption utilizing acetonitrile for the columns ELSIsphere® and Mikropak®, respectively. In both figure, it is clearly shown that, with the increasing flow rates of the mobile phase, the response of the both column becomes earlier. The area under the "C" curves was calculated and normalized to be unity [24]. In other words, the amount of acetonitrile adsorbed on the C18 surfaces was equal to zero. It was decided that, in this paper, the "C" curves obtained from the reference responses of the both columns were used for determining Peclet number to investigate the effect of axial dispersion.

The observed moments m_0^E, m_1^E and m_2^E were computed by numerical evaluation of the response peaks according to eqns. 10-12.

The Peclet number ($Pe_d = Ud_p/D$), which reflects the column flow characteristic in the case of no adsorption was calculated from eqn. 9 and the results are given in

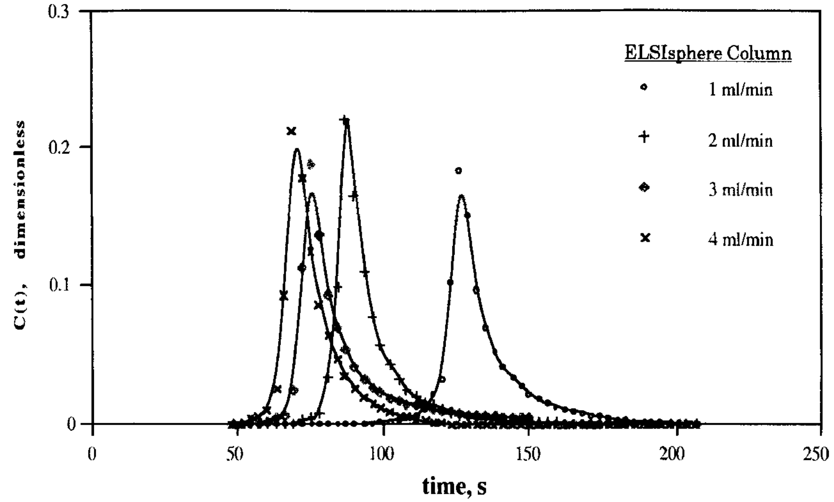

Figure 1. Response "C" curves of pulse-stimulated ELSIsphere® column at different flow rates

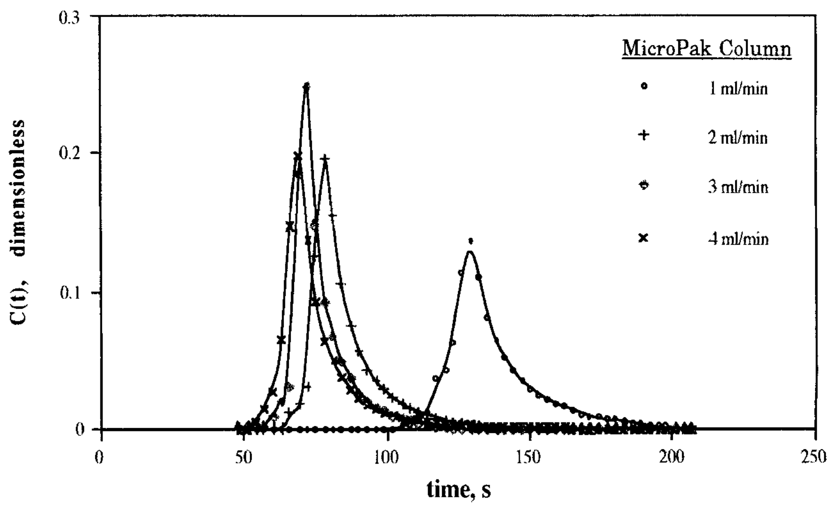

Figure 2. Response "C" curves of pulse-stimulated MikroPak® column at different flow rates

Table 1. Peclet Numbers (N_{Pe}) and Dispersion coefficients (D) of ELSIsphere® and Mikropak® C18 HPLC columns at different flow rates

	ELSIsphere®		Mikropak®	
Column Dimensions				
Length, L (mm)	150		150	
Inner Diameter, ID (mm)	4		4	
Packing Material	Octadecyl (C18)		Octadecyl (C18)	
Particle Size (μ)	5		5	
Flow Rate (ml/min)	N_{Pe}	D,cm^2/s x10^6	N_{Pe}	D,cm^2/s x10^6
1.0	158.8	3.86	141.9	4.12
2.0	109.4	8.07	93.2	10.16
4.0	48.1	22.21	46.6	23.03

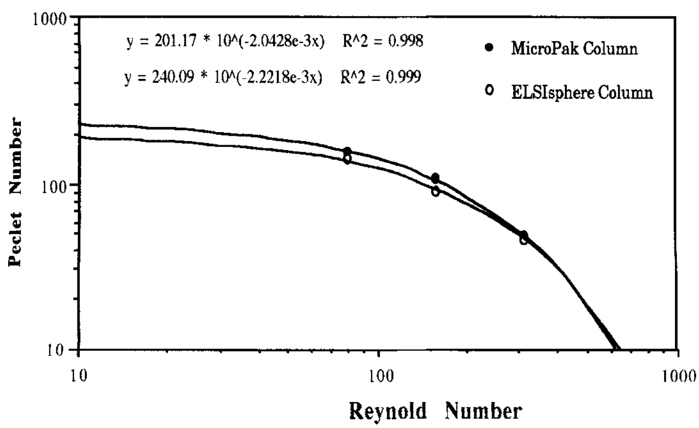

Figure 3. The change of Peclet Number via Reynold Number in C18 HPLC columns

the Table 1. Table 1 also includes the both column characteristics which are planned to be used in our future studies. When the results were compared with the literature, it is observed that, the trend of the curves drawn on the basis of Reynolds number , N_{Re} versus Peclet Number, N_{Pe}, (Figure 3.) are in a very good agreement with the literature [25-26]. It is important to note that calculated Peclet numbers for each column in this stage indicated a large amount of axial dispersion at these Reynolds numbers and the calculated values of axial dispersion coefficients, D, from those Peclet numbers (Table 1) directly characterized the effect of only axial dispersion in the column.

As conclusion, it can be said that, the stimulus-response may be considered as a deft dynamic approach to investigate the flow characteristics of fixed bed systems. The evaluation of 'C' curves by standard and cybernetic moment techniques gives the Peclet numbers and dispersion coefficients of the column, and further in case of utilizing interacting tracers; adsorption or absorption rate constants, affinity, diffusional, and other dynamic behavior of the columns would also be determined. Studies concerning the generalisation of this dynamic test approach are still under investigation [24, 27].

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