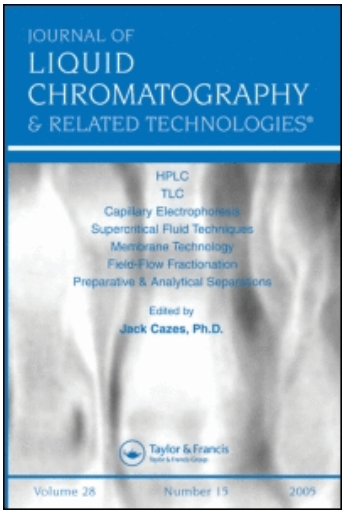

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DYNAMIC BEHAVIOUR OF C_{18} HPLC COLUMNS BY STIMULUS-RESPONSE ANALYSIS PART II: DETERMINATION OF DISPERSION COEFFICIENTS VIA PECLET NUMBERS

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ABSTRACT

In this study, the dispersion coefficients of acetonitrile, ascorbic acid, patulin, 5-hydroxymethylfurfural, 2,4-dichlorophenoxy- acetic acid, 4-chlorophenoxyacetic acid and ß-naphtoxyacetic acid are determined by using the stimulus-response technique. The system was pulse stimulated with 10 µL of each tracer with definite concentrations at the flow rate of 1 mL/min. The resulting chromatograms, so called response "C" curves, were obtained for each tracer, individually. The Peclet numbers of each tracer were calculated and evaluated to get dispersion coefficients. The relative dispersion coefficients, based upon the non interacting tracer Acetonitrile (D: 1.83×10^{-6} cm^2/s). were calculated as follows; acetonitrile: 1.00, ascorbic acid: 3.72, patulin: 1.34, 5-hydroxymethylfurfural: 1.68, 2,4-dichloro-phenoxyacetic acid: 2.32, 4-chlorophenoxyacetic acid: 3.23 and ß-naphtoxyacetic acid, 2.70.

INTRODUCTION

Stimulus-response methods have been well established in chemical engineering processes for measuring rate and equilibrium parameters such as mass transfer coefficients, diffusivities and adsorption rate constants.[1-3] The moments of the response curves to pulse inputs have been extensively used in the analysis of packed bed systems.[4-7]

The stimulus-response approach can also be used to investigate the dynamic interaction between mobile and stationary phases for high performance liquid chromatography (HPLC) systems. Two C_{18} HPLC columns have been examined using this technique, in order to compare their dynamic behaviours by us.[8]

Acetonitrile has been used as non-interacting tracer through the columns and than, Peclet number, which is the basic indication of the dynamic flow characteristic of the column and dispersion coefficients, have been determined.[8] The dispersion coefficients, calculated based upon the observed Peclet numbers, may give us an idea about how an analyte particle is retained in the column for a definite chromatographic condition.

In this study, the stimulus-response technique was employed to get the dispersion coefficients of acetonitrile, patulin, 5-hydroxymethylfurfural (5-HMF), ascorbic acid, 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chlorophenoxyacetic acid (4-CPA) and ß-naphtoxyacetic acid (BNOA). The column was pulse stimulated with 10 µL solution of each tracer and the responses of the column were measured individually.

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 for patulin and 5-HMF, 254 nm for ascorbic acid and 270 nm for 2,4-D, 4-CPA and BNOA to obtain the highest sensitivity. Varian model 4400 integrator was used with a chart speed of 2 cm/min to record the resulting chromatograms.

Columns

A column (ShimPak®) supplied from Shimadzu, made of stainless steel, 250 x 4 mm (id), packed with C_{18} octadecyl groups (5 μ), operated at ambient temperature, was used.

Mobile Phase

Mixture of water:acetontirile (95:5, v/v) filtered through a regenerated cellulose acetate membrane (0.45 μ) and degassed ultrasonically, was used as the mobile phase with the flow rate of 1.0 mL/min.

Test Tracers

Chemicals used were all obtained from Merck, Germany. Acetonitrile was HPLC grade and used without any further purification. Patulin and 5-HMF solutions were prepared in pH 4.0 water to a concentration of 2 μg/mL. Ascorbic acid solution, at a concentration of 10 μg/mL, was prepared dissolving an appropriate amount of ascorbic acid in water. It was then stabilised adding 1 mg of dithiothreitol for each mL of solution. 2,4-D, 4-CPA and BNOA solutions were prepared in a mixture of isopropanol:water (75:25, v/v) to a concentration of 1 μg/mL.

Stimulus-Response Analysis

The system was "pulse" stimulated by injecting 10 μl of acetonitrile as inert tracer or the other test tracers, separately, to the mobile phase at flow rate of 1 mL/min to determine the reference response of the column. Then, the column was stimulated with interacting tracers, individually. The column responses, which are so-called "C" curves, were determined by following the absorption of the tracers in the eluent stream. In each case, the absorption was detected by a UV-VIS detector. All the experiments were carried out at ambient temperature.

RESULTS AND DISCUSSION

The response "C" curves obtained in the stimulus-response experiments for the chemicals used in that group of study was given in Figure 1. It is clearly

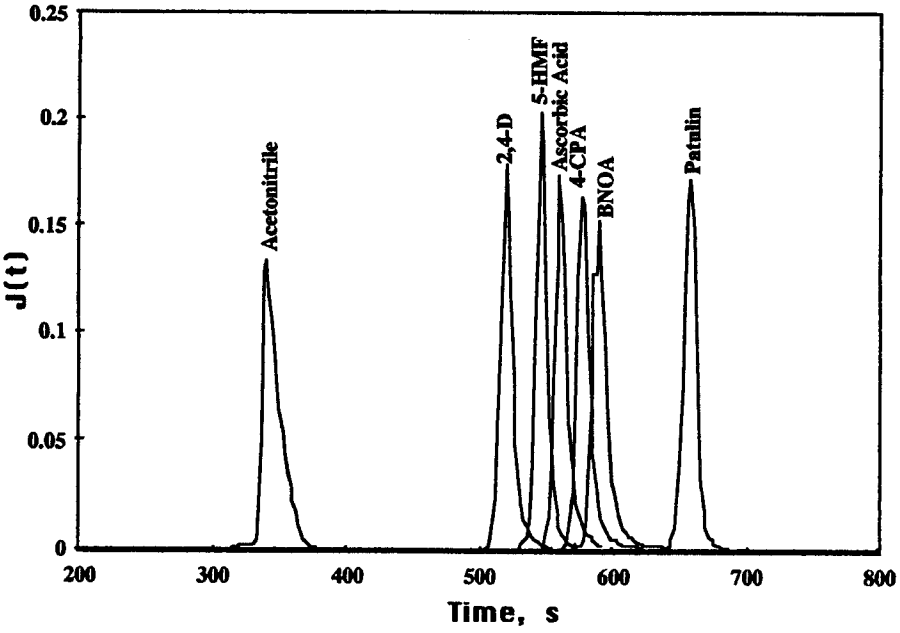

Figure 1. Response "C" curves of pulse-stimulated ShimPak® column for different chemicals

observed that, the non interacting tracer "acetonitrile" leaves the column first, than the others follow it, according to their interactive behaviour with both RP-HPLC column packing material and the mobile phase. It is well known that, due to interaction of chemicals with column packing material, the response of the column for a particular compound would delay, but such an interaction does not cause any loss of material. Therefore, the total amount of tracer injected to the column could be collected to obtain "C" curves. The area under the "C" curves was calculated and normalized to be unity, according to the analysis method given elsewhere.[9]

The principle mathematical analysis method, so called "parameter estimation by cybernetic moment technique", to obtain the individual Peclet numbers, N_{Pe} from "C" curves are given in our previous paper.[8] The equation describing the Peclet number is:

$$N_{Pe}=\frac{U\,d_p}{D} \qquad (1)$$

where:

N_{Pe} : Peclet number, dimensionless
U : interstitial velocity, cm s^{-1}
d_p : particle diameter, cm
D : dispersion coefficient, cm^2s^{-1}

The response "C" curve which belongs to each chemical, was evaluated to obtain Peclet numbers, and by utilising Equation 1, the dispersion coefficients were calculated. The relative dispersion coefficients based upon the non interacting tracer Acetonitrile was also calculated, and the results are given in Table 1.

It is very well known that, Peclet number is the ratio of the mass transfer by bulk flow to mass transfer by diffusion. The non interactive tracer "acetonitrile" is carried through the column by the mobile phase, but due to its' non interactive behaviour with column packing material, the dispersion through the pores becomes less effective in transportation of molecule. Therefore, the dispersion coefficient of acetonitrile ($D=1.83x10^{-6}$ cm^2/s) is found as the minimum of all others. Patulin, which is a mycotoxin and 5-HMF have similar chromatographic properties, and therefore, separation of patulin from 5-HMF is difficult as recorded in the literature.[10-11]

The dispersion coefficients of patulin and 5-HMF are found to be $2.45x10^{-6}$ cm^2/s and $3.08x\ 10^{-6}$ cm^2s, respectively. Those values are closed to each other which confirms the difficulty of separation. 2,4-D, 4-CPA and BNOA, which belong to a group of chemicals called "phenoxycarboxylic acids", have also shown similar interactive behaviours with the column packing material as expected, due to their similar aromatic chemical structures. It is thought, that the separation of these acidic compounds may not be easy using a common solvent mixture like water:acetonitrile (95:5, v/v) as can be seen easily from their dispersion coefficients and response "C" curves in Figure 1.

The dispersion coefficient of the final compound, ascorbic acid, was found to be $6.80x10^{-6}$ cm^2/s and that value is considerably greater than that of phenoxycarboxylic acids'. This can be explained by the molecular structure of ascorbic acid which extends polarity of molecule.

As conclusion, it can be said that, the stimulus-response analysis of several compounds to determine their mass transfer behaviour through the RP-HPLC column may give a brief idea about the separation efficiency of the column for a defined chromatographic condition. The preestimation of the

Table 1

Peclet Numbers (N_{pe}), Dispersion Coefficients (D) and Relative Dispersion Coefficients (Rel D) for Various Chemicals

Tracer	N_{pe} (Dimensionless)	D cm^2/s	Rel D (Dimensionless)
Acetonitrile	36.259	1.83×10^{-6}	1.00
Patulin	27.077	2.45×10^{-6}	1.34
5-hydroxymethylfurfural	21.529	3.08×10^{-6}	1.68
2,4-dichlorophenoxylacetic acid	15.645	4.24×10^{-6}	2.32
β-naphtoxyacetic acid	13.420	4.94×10^{-6}	2.70
4-chlorophenoxyacetic acid	11.225	5.91×10^{-6}	3.23
Ascorbic acid	9.746	6.80×10^{-6}	3.72

separability of some complex mixtures can be investigated by suggested technique. Studies concerning the generalization of this dynamic test approach are still under investigation.

REFERENCES

1. G. Doğu, J. M. Smith, AlChE J., **21(1)**, 58-61 (1975).

2. V. Kafarov, **Cybernetic Methods in Chemistry and Chemical Engineering**, Mir Publishers, Moscow, 101-123, 1976.

3. G. Doğu, **Handbook for Heat and Mass Transfer Operations**, N. P. Cheremisinoff, Gulf Publ. Co., 401-448, 1986.

4. P. Schneider, J. M. Smith, AIChE J., **14**, 762 (1968).

5. N. Hashimoto, J. M. Smith, Ind. Eng. Chem. Fund., **12**, 353 (1973).

6. H. W. Haynes, Jr., P. N. Sarma, AIChE. J., **19**, 1043-1047 (1981).

7. L. K. P. Hsu, H. W. Haynes, Jr., AIChE. J., **27**, 81-90, (1981).

8. M.Mutlu, V. Gökmen, J. Acar., J. Liq. Chromatogr., **18(9)**, 1747-1755 (1995).

9. M. Mutlu, E. Pişkin, Med. Biol. Eng. Comput., **28**, 232-236 (1990).

10. J. Acar, V. Gökmen, Flüss. Obst., **62(8)**, 368-370 (1995).

11. V. Gökmen, J. Acar, J. Chrom. A, (1996) (in press).

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