Journal of Chromatography, 202 (1980) 233–237
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,283

PULSED INJECTIONS OF ION INTERACTION REAGENT APPLIED TO THE LIQUID CHROMATOGRAPHIC SEPARATION OF 2,6-DISUBSTITUTED ANILINES

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(First received July 22nd, 1980; revised manuscript received August 20th, 1980)

SUMMARY

The effect of the number of pulsed 20-μl injections of 10 mM octanesulfonate on the retention times of aniline and five 2,6-disubstituted anilines was determined by fitting a model derived from the Freundlich adsorption isotherm to data collected at six different numbers of pulses. An optimization strategy based on the window diagram technique of Laub and Purnell predicted that approximately 40, 20-μl pulses of 10 mM octanesulfonate would give optimum separation using a methanol–water (45:55) mobile phase. These conditions gave baseline separation of the six components.

INTRODUCTION

Reversed-phase high-performance liquid chromatography has been highly successful in the separation and quantitation of mixtures of chemically similar compounds. Good separation can usually be achieved by judicious selection of the mobile phase composition with respect to pH, percent methanol, concentration of ion interaction reagent (IIR), etc. It is then often desireable to optimize the separation by fine adjustment of the eluent composition to aid in the quantitation of closely eluting compounds.

Laub and Purnell[1] have shown that plotting separation factor (α) curves, for all possible pairs of compounds in a mixture, *vs.* a variable chromatographic factor produces a "window diagram" that can be used to optimize a separation with respect to the variable factor.

Optimum separation is obtained when the variable factor is adjusted to the value corresponding to the top of the tallest "window" in the α plot.

In this paper we describe a method for increasing the separation power of an isocratic system by using pulsed injections of an IIR (octanesulfonate) as a variable chromatographic factor. We also give a mathematical model describing the retention behavior of the compounds studied as a function of the number of pulses of IIR and show the use of window diagrams to optimize the separation obtained with the pulse technique.

0021-9673/80/0000–0000/$02.25

THEORETICAL

The effect of an IIR on the retention behavior of both positively and negatively charged compounds has been investigated previously by Bidlingmeyer *et al.*[2]. They proposed an ion interaction model in which the increased or decreased retention of a solute ion is caused by the amount of charge in the primary ion layer arising from the adsorption of IIR on the stationary phase. The amount of adsorbed IIR is a function of the concentration of IIR in the mobile phase and can be described by the Freundlich adsorption isotherm[3]. Thus the capacity factor of the solute as a function of [IIR] can be described by

$$k' = \beta_0 + \beta_1 [\mathrm{IIR}]^{1/\beta_2} \quad (1)$$

where k' is the capacity factor of the charged solute, β_0 is the capacity factor in the absence of IIR, β_1 is a parameter describing the "effectiveness" of the IIR, and β_2 is a parameter of the Freundlich isotherm. We have found that when [IIR] in eqn. 1 is replaced by the number of pulses of IIR, an excellent fit is also obtained.

$$k' = \beta_0 + \beta_1 n^{1/\beta_2} \quad (2)$$

where n is the number of injections of IIR.

EXPERIMENTAL

Chromatographic system

The chromatographic system consisted of a Model 6000A solvent delivery system, a 5 cm × 4 mm I.D. Bondapak precolumn and a 30 cm × 4 mm I.D. μBondapak C_{18} main column, all from Waters Assoc. (Milford, MA, U.S.A.). An electrochemical cell equipped with glassy carbon electrodes (Bioanalytical Systems) was used in the amperometric mode at +0.9 V *vs.* an Ag/AgCl reference electrode. A computer controlled Model 70-10 automatic sample injection valve equipped with a Model 70-01 pneumatic activator (Rheodyne) was used to inject 20-μl volumes of IIR. A Model U6K variable volume injector (Waters Assoc.) was used for the introduction of samples downstream from the Rheodyne injector. Precolumn and main column temperatures were maintained at 25.0 ± 0.1°C by a Model FK constant-temperature circulating bath (Haake). The flow-rate was set at 2.0 ml/min and the time equivalent of the void volume (t_0) was 1.697 min.

Additional instrumentation

The analog electrochemical detector output was recorded by a Model 281 stripchart recorder (Soltec). Simultaneouly the signal from the detector was digitized by a Model ADC-12QZ analog-to-digital converter (Analog Devices) interfaced to a Model 9830A computer (Hewlett-Packard). The 9830A also controlled the Rheodyne injector.

Mobile phase and samples

The mobile phase consisted of methanol–water (45:55) containing 1 m*M* HCl.

A sample mixture[4] approximately 0.2 m*M* in aniline, 2-methyl-6-isopropylaniline (MIPA), 2-ethyl-6-isopropylaniline (EIPA), 2,6-diisopropylaniline (DIPA), 2-methyl-6-*tert.*-butylaniline (MTBA) and 2-ethyl-6-*sec.*-butylaniline (ESBA) (Ethyl Corp., Baton Rouge, LA, U.S.A.) was prepared in the mobile phase.

Pulsing of IIR

10 m*M* octanesulfonate (Eastman-Kodak, Rochester, NY, U.S.A.) was made up in the mobile phase. The IIR was pumped continuously through the Rheodyne valve by a peristaltic pump at approximately 3 ml/min. A specified number of 20-μl injections of IIR were made at 5-sec intervals under computer control. During an injection of IIR the Rheodyne valve was left in the inject mode for one second and then returned to fill mode. The samples were injected manually with the Waters Assoc. injector. After each chromatogram, 100% methanol was run through the column for 2 min followed by eluent for approximately 3 min before the next injection.

Experimental design

A six-level experimental design corresponding to 0, 5, 10, 20, 40 and 60 pulses, with replicates at 0 and 20, was used. The experimental order was randomized to minimize the confounding of time trends with factor effects.

RESULTS AND DISCUSSION

Fig. 1 shows a set of chromatograms obtained at 0, 5, 10, 20, 40 and 60 pulses of IIR. In the chromatogram at 40 pulses, the elution order is aniline, MIPA, EIPA,

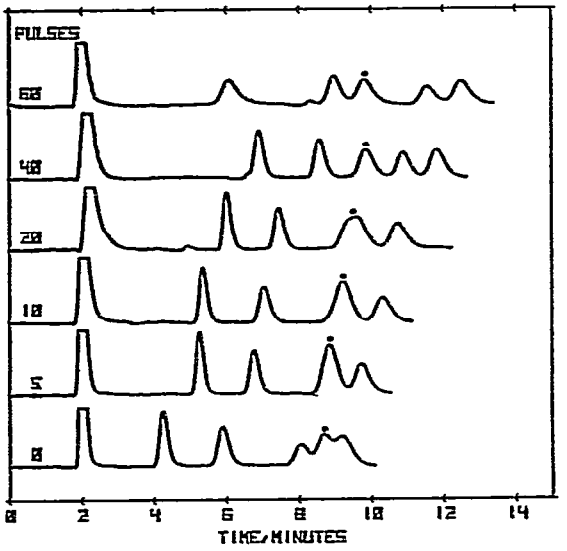

Fig. 1. Chromatograms of 10-μl injections of a mixture 0.2 m*M* in each of aniline, MIPA, EIPA, MTBA, DIPA and ESBA obtained at 0, 5, 10, 20, 40 and 60 pulses of octanesulfonate, 5 sec between pulses. Dot indicates elution of MTBA.

MTBA, DIPA and ESBA. It can also be seen in Fig. 1 that increasing the number of pulses of IIR gives a regular increase in retention of all components except MIPA at 60 pulses.

Table I contains the best non-linear least squares estimates of the parameters of eqn. 2 for each of the six samples. The small standard deviations of residuals show a good fit of the model to the data.

Fig. 2 shows a window diagram which plots the predicted separation factors (α) of all possible pairs of compounds as functions of the number of pulses. Separation

TABLE I

CAPACITY FACTORS AND PARAMETERS ESTIMATES

Pulses	*Capacity factors*					
	Aniline	*MIPA*	*EIPA*	*MTBA*	*DIPA*	*ESBA*
0	0.178	1.50	2.49	4.17	3.77	4.43
5	0.197	2.15	3.05	4.30	4.30	4.83
10	0.245	2.21	3.23	4.53	4.53	5.18
20	0.289	2.62	3.54	4.71	4.82	5.47
40	0.294	3.15	4.15	4.94	5.54	6.10
60	0.215	2.60*	4.39	4.53	5.94	6.51
	Parameter estimates					
β_0	0.178	1.51	2.49	4.15	3.78	4.43
β_1	0.047	0.237	0.226	0.128	0.171	0.165
β_2	6.18	1.92	1.90	2.182	1.61	1.61
S_r**	0.049	0.074	0.078	0.093	0.082	0.064

* Not included in least squares fit.
** Standard deviation of residuals.

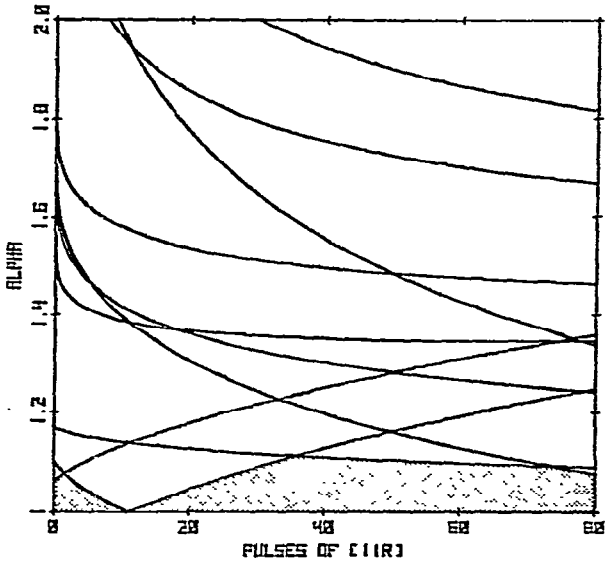

Fig. 2. α plot for the six anilines showing optimal separation conditions at 36 pulses of octanesulfonate.

factors greater than 2 are not shown. The minimum at approximately 10 pulses shows conditions where compounds coelute. The window diagram predicts optimal separation at 36 pulses; thus the excellent separation in the chromatogram at 40 pulses in Fig. 1 is very close to the optimal separation attainable with this system.

In the chromatogram at 60 pulses (see Fig. 1), MIPA elutes earlier than predicted by the trend in the other experiments. We believe this anomalous result is caused by coelution of the sample with the last of the IIR pulses. We have observed this sudden shift in retention behavior in other pulse studies; the mechanism for this behavior is under current investigation.

CONCLUSION

This study has shown that pulsed injection of IIR is an effective technique for improving separation in isocratic liquid chromatography. One advantage of the pulsed technique over conventional "ion-pair" chromatography (in which the IIR is constantly present in the eluent) is that a lengthy column equilibration period at the beginning of a set of chromatographic runs is not necessary to obtain reproducible retention times. Another advantage is forseen in the separation of a complex mixture containing positively charged, negatively charged and uncharged components: the timing and duration of the pulses can be adjusted so that the retention time of components with the same charge as the IIR would be adequately decreased, and the retention time of oppositely charged components would be adequately increased to give the desired separation[2].

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