Feasibility of Enhancing High-Performance Liquid Chromatography Using Microwave Radiation

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Abstract

High-performance liquid chromatography (HPLC) is the most versatile of the chromatographic techniques because it is applicable to a wide variety of analytes. Unfortunately, liquid mobile phases have relatively low diffusivities. A novel approach is presented for increasing the diffusivity of a liquid mobile phase. The method advocated in this study is the first process that has been described by which it is possible to increase the diffusivity of a liquid while having essentially no effect on other physical properties of the liquid, such as the temperature or solvating power. It is demonstrated that it is possible to sharpen peaks, in an HPLC separation, by the application of microwave radiation.

Introduction

It is well known that the diffusivity of the mobile phase is a critical parameter in chromatographic separations. The speed or efficiency-per-unit time of the three major chromatographic methods increases in the order: high-performance liquid chromatography (HPLC) < supercritical fluid chromatography (SFC) < gas chromatography (GC). The reason is that the diffusivities of the respective mobile phases increase in the same direction (1). The relative speeds of chromatographic methods were first discussed by Giddings (1) in 1965.

If we consider the solvating power of the mobile phase, the trend would be exactly the opposite of that previously mentioned. Therefore, although GC is a fast technique, the mobile phase has essentially no solvating power and thus only analytes that possess some volatility can be chromatographed. SFC has a wider range of applicability because the mobile phase does in fact possess a degree of solvating power. However, HPLC is the most versatile of the three techniques because liquids have, by far, the greatest ability to solvate analytes.

If the diffusivity of a liquid mobile phase were to be increased, the result would be a technique that offers the best of both worlds. The elevated diffusivity would allow for faster analysis and the high solvating power of the liquid would make it possible to chromatograph virtually any type of analyte. Previously, this has been accomplished by conducting liquid chromatography (LC) at highly elevated temperatures (2–6). Most of this work has focused on open-tubular column LC (2,3) or separations with packed capillaries (4,5). Recently, the use of elevated temperature was applied to a 4.6-mm-i.d. packed column (6). Using temperatures up to 150ºC, analysis times were reduced by a factor of 50. However, there were several disadvantages to this approach. First, the mobile phase needed to be preheated prior to reaching the column by passing it through a certain length of tubing. Unfortunately, there was a delay associated with this. In order for fast analysis to still be obtained, the analytes were delivered by a separate line (with a narrower i.d.) that fed into the mobile phase line immediately upstream of the column. Thus, there was a significant dilution of the analytes resulting in a loss of sensitivity. Secondly, additional hardware was required in order to maintain pressure in the column (so that the solvents did not boil at the elevated temperatures) as well as to cool the mobile phase down prior to reaching the detector. Thirdly, with this technique the analyst is limited in the types of stationary phases that can be used. Polystyrene-coated zirconia phases were primarily used by Yan et al. Lastly, this approach is problematic if working with analytes that are not temperature stable.

An alternative approach to elevating the diffusivity of a liquid mobile phase was used in this study, which made use of the phenomenon of microwave-induced dielectric polarization. Electromagnetic radiation of any kind consists of an electric field and a magnetic field at right angles to one another and continually increasing and decreasing in magnitude. The dielectric polarization phenomenon can be understood by considering that when the electric field increases in size, molecules that contain a dipole are caused to align with the field. When the electric field subsides, the molecules randomize (7). At 2450 MHz (the frequency typically used in household and laboratory microwave systems) this alignment and randomization occurs 4.9 billion times per second (7).

Although this phenomenon is often used for heating liquids (8), it is possible to increase the diffusivity of a liquid while having only a minimal effect on its temperature by pulsing with very short "bursts" of microwave radiation. In fact, this method is the only approach (as far as we are aware) by which it is possible to

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increase the diffusivity of a liquid while having virtually no effect on other physical properties of the liquid (most notably its temperature or solvating power). Thus, it is possible to reap all the resultant advantages of a high-diffusivity mobile phase while avoiding the difficulties that result from the use of highly elevated temperatures.

Experimental

Several analyses were undertaken in order to demonstrate that the dielectric polarization phenomenon could, in fact, be used to sharpen chromatographic peaks in an HPLC separation. A microwave capable of delivering very short pulses of radiation was not available; therefore, for purposes of preliminary evaluation, a Samsung domestic 600-W microwave oven (Ridgefield Park NJ) at the standard 2450 MHz frequency was used. Because this system is not capable of any type of power programming, it was manually turned on and off throughout the analysis. A Hewlett-Packard (Wilmington, DE) Model 1050 HPLC pump was used along with a Model 1050 autosampler and a Model 1050 variable wavelength UV detector. Data were collected on a Hewlett-Packard Model 3394A integrator.

The mobile phase was composed of methanol and water with no buffers or additives. The gradient used was 70:30 methanol–water for 1.5 min ramped to 90:10 methanol–water at 7 min and held there for the remainder of the run. For all analyses, 10 µL was injected, a flow rate of 1 mL/min was used, and a wavelength of 205 nm was set for detection. A 25-cm IonPac NS-1 column with a 4-mm i.d. was obtained from Dionex (Sunnyvale, CA) and used for all analyses. The column contained 5-µm polystyrene divinylbenzene particles. The polystyrene was cross-linked (55%) with divinylbenzene. The stationary phase, which is marketed for biomolecular applications, contained both micropores and macropores. Because this column was designed for ion chromatography, it was made of poly-ether-ether-ketone rather than metal. This was important because metal would have been problematic in the microwave oven.

The stock solution used contained 2-naphthol at 307 ng/µL and benzene at 566 ng/µL in methanol, which meant that approximately 3 µg of 2-naphthol and 6 µg of benzene were introduced to the column for each separation. The column was positioned in the microwave oven at a slight angle, but was more horizontal than vertical. Care was taken to keep the column off the oven floor and away from the walls. In other words, the column was suspended in mid-air.

Results and Discussion

The plate number was used as a quantitative measure of efficiency. This was determined by taking the height-to-area ratio (h/A) calculated by the integrator and plugging it into the following formula in order to obtain theoretical plates (N):

$$N = 2\pi\,(h/A \times t_R)^2 \qquad \text{Eq. 1}$$

The application of radiation was delayed for 1 min and 20 s from the start of the run in order to first allow the analytes to reach the head of the column. The data showed that when short pulses (approximated at $^1/_3$ of a second) were applied at 10-s intervals, an increase in the plate count resulted. When these pulses were applied more closely together, the effect was considerably more pronounced. The data are presented in Table I with the retention times of the peaks included in parentheses. The enhancement in the chromatographic traces is shown in Figure 1. The chromatograms are aligned so that the peak axes are in the same

Table I. Plate Count as a Function of the Microwave Pulse Interval

	Plate count t_R	
	2-Naphthol	Benzene
Microwave off	2493 (12.8)	2492 (15.5)
~ $^1/_3$ of a second pulse every 10 s	2921 (12.4)	2545 (15.0)
~ $^1/_3$ of a second pulse every other second	4217 (10.6)	3260 (12.3)
% Increase (pulse every other second vs. no radiation)	69%	31%

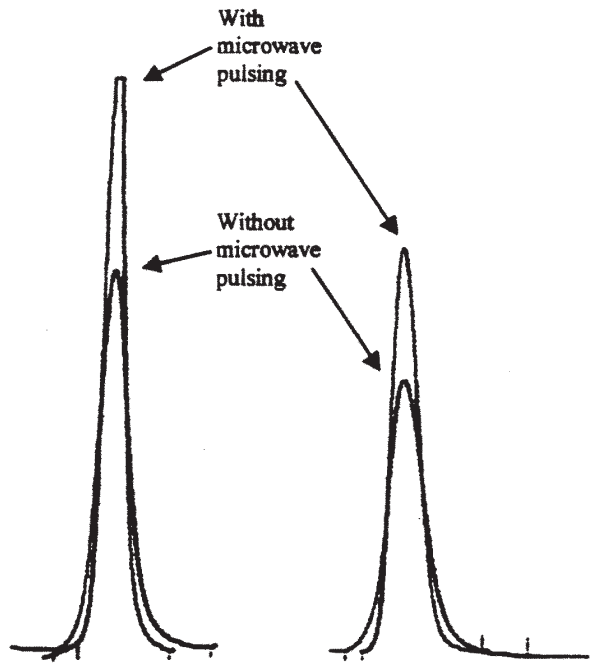

Figure 1. The peaks on the left are 2-naphthol, and the peaks on the right are benzene.

Table II. Effect of Heating on Plate Count

	Plate count t_R	
	2-Naphthol	Benzene
Column ambient	2493 (12.8)	2492 (15.5)
Column in oven at 75°C	2070 (10.7)	2061 (12.6)
%Decrease versus no radiation or heat	17%	17%

place. The two chromatograms have slightly different retention times.

Because of the crude nature of the system used, it was impossible to deliver the very short pulses of radiation that would have been ideal for this application. As a result, some heating of the mobile phase did occur because one could feel the column getting warm. In order to confirm that the sharpening of the peaks was a result of the dielectric polarization phenomenon (and not the temperature change), the column was placed in a conventional oven and heated. The data in Table II were obtained with the oven set at 75°C (the actual temperature of the mobile phase was undoubtedly lower than this, but this setting resulted in retention times very similar to those obtained in the run in which pulsing was applied every other second, thus indicating that the temperature of the mobile phase was comparable). The plate counts obtained were somewhat lower in comparison with the run with no heating or radiation. The increase in plate count with microwave pulsing compared with the slight decrease in plate count when heated in a conventional oven provided clear evidence that the dielectric polarization phenomenon was the primary cause of the improved efficiency. The observed negative effect of conventional heating on column efficiency was consistent with previous studies (9–13) and attributable to the thermal gradients that arise when the temperature of the mobile phase (as it enters the column) is not equivalent to the temperature of the column itself.

Conclusion

In this study, it has been demonstrated that it is possible to sharpen peaks in HPLC by applying microwave radiation. There can be little question that much shorter pulses would have been ideal because they would have allowed the radiation to be applied with sufficient regularity so that the maximum effect could be obtained yet simultaneously minimize heat generation in the column. Along this line, it is believed that what has been demonstrated in this study is only a small fraction of the true potential of the technique. It is, however, encouraging that a significant improvement was observed despite the use of a very crude microwave system.

Work is currently underway to demonstrate the full potential of the technique. This, along with a thorough discussion of relevant theoretical issues, will be published subsequently.

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