Comparison of chromatographic band profiles obtained under microwave irradiated and non-irradiated reversed-phase liquid chromatography column

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Abstract

The possible influence of the application of microwave energy to a reversed-phase liquid chromatography column on the mass transfer kinetics and the thermodynamics of equilibrium between mobile and stationary phases was examined. Chromatograms of propylbenzene and phenol were recorded under the same experimental conditions, on the same column, successively irradiated and not. The effect of microwave irradiation on the mass transfer kinetics was determined by measuring the second moment of small pulses of propylbenzene in a 70:30 (v/v) solution of methanol in water and microwave outputs of 15 and 30 W. The effect of microwave irradiation on the equilibrium thermodynamics was determined by measuring the elution time of breakthrough curves of phenol at high concentrations in a 20:80 (v/v) solution of methanol and water and microwave outputs of 15, 50, and 150 W. A qualitative comparison of the profiles of the propylbenzene peaks obtained with and without irradiation suggests that this irradiation affects significantly the peak shapes. However, a qualitative comparison of the profiles of the breakthrough curves of phenol obtained with and without irradiation suggests that this irradiation has no significant effect on their shapes. The peak sharpening observed may be due to an increase in the diffusivity, resulting from the dielectric polarization under microwave irradiation. This effect is directly related to an increase of the rate of mass transfers in the column. In contrast, the similarity of the overloaded band profiles at high concentrations suggests that the equilibrium thermodynamics is unaffected by microwave irradiation. This may be explained by the transparence of the stationary phase to microwaves at 2.45 GHz. The column temperature was measured at the column outlet under irradiation powers of 15, 30, 50, and 150 W. It increases with increasing power, the corresponding effluent temperatures being 25 ± 1, 30 ± 1, 35 ± 1, and $45 \pm 1\,^\circ$C, respectively.
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1. Introduction

In recent years, microwave (MW) dielectric heating has become an established procedure in synthetic chemistry [1–4]. However, its application to various chemical reactions is still accompanied by speculations on the influence of microwave irradiation on the reaction rates. There are reports of chemical reactions that proceed faster in a microwave environment than under conventional conditions at the same temperature. Factors between five and over one thousand have been reported for the enhancement of the reaction rate of the same system in an irradiated environment over a non-irradiated one [5–8]. Other reactions were reported to proceed at the same rate under microwave irradiation and under conventional conditions. Both irradiated and non-irradiated reactions are governed by the same fundamental principles of thermodynamics and kinetics [9–11]. All previous reports, however, are derived from the study of chemical reactions performed in batch or static conditions. The effect of microwave irradiation on chemical reactions taking place in continuous flow reactors, such as in chromatographic

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columns or more generally in liquid–solid separations, is yet to be investigated, although a positive effect of MW irradiation has been suggested [12]. This effect was not isolated from the consequences of MW heating of the mobile phase, however, and more detailed investigations are warranted

Chromatographic separations involve a liquid–solid system, and analyte diffusivity in the mobile phase is a critical parameter controlling column efficiency. Increasing this diffusivity allows for faster analyses. The conventional approach is conducting liquid chromatography at high temperatures [13–17]. Operating a column at a temperature of 150 °C permits a reduction of the analysis time by a factor of 50. However, this approach has several drawbacks, e.g., the need to pre-heat the mobile phase before it enters into the column, the limited number of stationary phases that are thermally stable, and the possibility of thermal decomposition of some analytes. The use of microwave dielectric polarization offers a possible way to increase the diffusivity of analytes in the mobile phase.

Microwaves interact with molecules depending on their dielectric properties. The dielectric constant indicates the ability of a material to be polarized by an electric field, e.g., a microwave field. The polarization of dielectrics arises from the finite displacement of charges or the physical rotation of dipoles in an electric field. Thus, molecules that contain a dipole tend to align with the field when this field varies. When the electric field subsides, the molecules orientation randomizes [1,18]. Alignment and randomization take place at 4.9 GHz [18]. However, the dielectric constants of microwave-active molecules widely vary. For example, the dielectric constants of water, methanol, and propylbenzene are 78.54, 32.63, and 2.3 at 25 °C, respectively. It is 9.78 at 60 °C for phenol [19]. Pure silica has a dielectric constant close to zero; it does not heat appreciably when exposed to microwave irradiation and it is considered as transparent to microwave radiations [20].

Recently, the feasibility of applying microwave irradiation in high-performance liquid chromatography was demonstrated by irradiating a column with short pulses, using a non-programmable, domestic-type 600-W microwave oven at 2.45 GHz [12]. The shapes of peak profiles obtained with and without irradiation were compared. An enhancement of the efficiency of unretained peaks under microwave irradiation was shown. The sharpening of the peaks was taken as a consequence of an increase in the diffusivity of the mobile phase.

The purpose of our work was to determine whether microwave irradiation affects significantly the kinetics of the mass transfers in reversed-phase liquid chromatography. Specifically, this work was to investigate under which experimental conditions could MW irradiation be used to enhance chromatographic separations since microwaves have selective and complex interactions with materials. Our goal was not to investigate the potential advantages of MW heating of the column versus the more conventional approaches that are used to operate HPLC columns at high temperatures. From this viewpoint, MW will always have the major disadvantage of generating an important temperature gradient along the column. In this study, heating was considered as a parasitic effect, impossible entirely to avoid and difficult to correct for. We purposefully used low output power to limit its direct effects.

2. Experimental

2.1. Chemicals

The mobile phases used were solutions of HPLC grade water and methanol (70:30 and 20:80 methanol/water, v/v). Both solvents were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Phenol and thiourea were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and propylbenzene was obtained from Aldrich Chemical Co. (Milwaukee, WI, USA).

2.2. Materials

Metals cannot be used in a microwave oven. The column tube was made of polyetheretherketone (PEEK). A manufacturer-packed, 150 mm × 4.6 mm, Luna $C_{18}(2)$ column with an average particle size of 50 μm (Phenomenex, Torrance, CA, USA) was used for all chromatographic measurements. Luna $C_{18}(2)$ is made of spherical particles of porous silica, chemically bonded with octadecylsilane, and endcapped. The large particle diameter was selected in order to magnify the effect of the resistance to the mass transfer across the particles.

2.3. Apparatus

The microwave oven system was an Ethos solvent extraction labstation (Ethos E, Milestone Inc., Shelton, CT, USA). It is a rugged, heavy-duty professional microwave unit designed for laboratory uses. It has three pre-fabricated holes through the top of the oven intended for continuous flow reactions. It has an original microwave feed/distribution system to the working cavity, fed by two 800-W industrial magnetrons operating at 2.45 GHz, each one fitted with its own high voltage power supply. The total system power is limited by design to 1000 W to ensure a long magnetron life. The microwaves are first fed into a premixing chamber from where a pyramid-shaped rotating metal diffuser evenly distributes the microwaves into the cavity. This unique design and its microprocessor control ensure optimal microwave distribution in the cavity, thus prevent localized "hot spots". The microwave unit includes a separate touch-screen controller with built-in software for advanced method development, archiving, and retrieval. It features a real-time graphical display of the parameters, such as the microwave power output, the temperature, the time, and the pressure, which can be modified before and during the operation. The

microwave power applied to the column was varied from 15 to 150 W.

An HP 1100 series (Hewlett-Packard, Palo Alto, CA, USA) was employed for all chromatographic measurements. The instrument is equipped with a pump, a thermostat, a diode array UV-spectrometric detector, and a computer data station that controls its operation. A Rheodyne manual sample injector (model 7725(i)) with a standard 20-μL sample loop (Rohnert Park, CA, USA) was used for the pulse injections. To generate breakthrough curves, a 5-mL sample loop was used. The signals of propylbenzene and phenol were detected at wavelengths of 254 and 291 nm, respectively.

A thermocouple, Atkins VersaTuff Plus Model 39658, type K (Tech Instrumentation, Parker, Co., USA) was used to measure the column effluent temperature. It has a temperature range from -40 to $1000\,^{\circ}\text{C}$, with an accuracy of $\pm 0.5\,^{\circ}\text{C}$ between -40 and $257\,^{\circ}\text{C}$. The thermocouple probe has a $178\,\text{mm} \times 2\,\text{mm}$ bendable stainless steel tip with a 1 s response time.

2.4. Chromatographic conditions and measurements

The chromatographic column was placed inside the microwave oven in a hanging position, i.e., suspended in mid-air, supported by the PEEK connecting tubings at its inlet and outlet. The inlet tubing connects the manual injector to the column and the outlet tubing connects the column to the detector. The thermocouple was placed against the tubing evacuating the eluate stream in order to continuously monitor and measure the temperature of the effluent leaving the column.

Pulses of propylbenzene (20 μL, 1.0 and 1.5 g/L) were injected in the mobile phase (flow rate 1.2 mL/min, composition 70:30 methanol/water), with microwave power outputs of 0, 15 and 30 W. Breakthrough curves of phenol were obtained by pumping into the column solutions of phenol at 10 to 50 g/L in the mobile phase (flow rate, 1.2 mL/min, composition 20:80 methanol/water), with microwave power outputs of 0, 15, 50, and 150 W. The chromatograms of propylbenzene and phenol were measured and recorded under the same experimental conditions and temperature, on the same column, using the same HPLC accessories (injector, detector, column fittings) with and without microwave irradiation. To obtain accurate results, the column was equilibrated for at least an hour prior to any measurements when the power output of the magnetrons was changed.

The first absolute moment (μ_1) and the second central moment (μ_2') of the elution peaks were calculated using the built-in program of the HP ChemStation. The start and end points of the peaks were automatically selected, based on the detection of the signal slope threshold. The contributions of the second moment arising from the injection of the probe compound into the stream of mobile phase and from the axial dispersion in the extra-column volumes of the instrument were subtracted from the second central moment of the solute.

The extra-column volume from the injection valve to the column and from the column to the detector was derived from the retention volumes of the solute, the column being replaced with a zero-volume connector. This volume was subtracted from the first moments of the bands.

3. Results and discussion

3.1. Comparison of chromatograms obtained under dilute conditions

Figs. 1–4 show elution peaks of propylbenzene obtained with and without MW irradiation at 15 and 30 W, respectively.
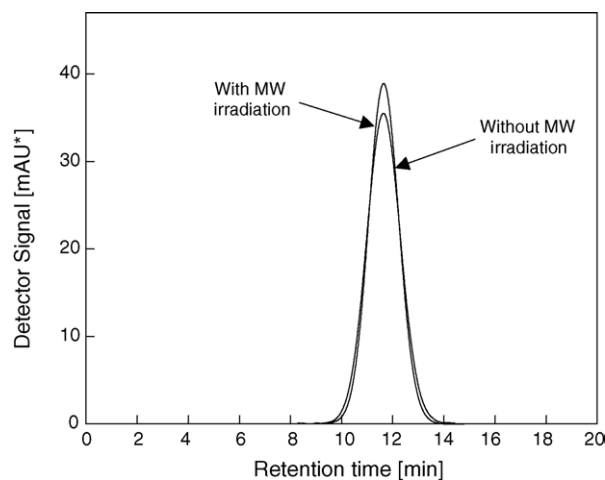

Fig. 1. Comparison between the experimental band profiles of propylbenzene obtained with and without microwave irradiation with a corresponding effluent temperature of $25 \pm 1\,^{\circ}\text{C}$ for both peaks (concentration: 1.0 g/L; injection volume: 20 μL; microwave power output: 15 W; mobile phase: 70:30 (methanol/water); flow rate: 1.2 mL/min).
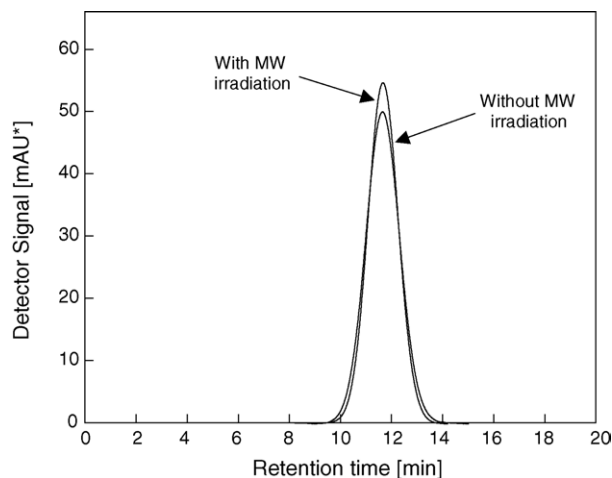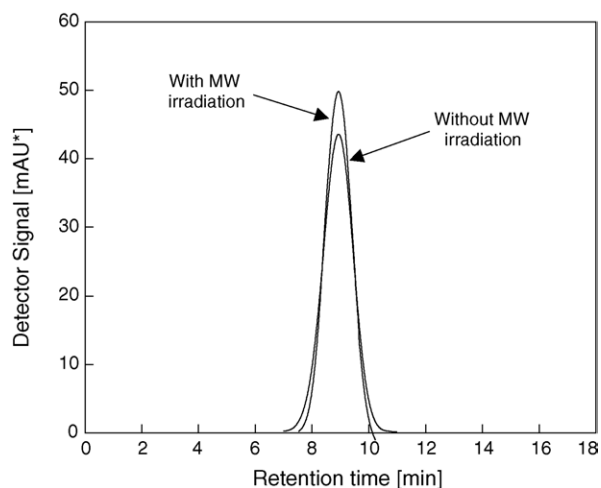

Fig. 2. Comparison between the experimental band profiles of propylbenzene obtained with and without microwave irradiation, with a corresponding effluent temperature of $25 \pm 1\,^{\circ}\text{C}$ for both peaks (concentration: 1.5 g/L; injection volume: 20 μL; microwave power output: 15 W; mobile phase: 70:30 (methanol/water); flow rate: 1.2 mL/min).

Fig. 3. Comparison between the experimental band profiles of propylbenzene obtained with and without microwave irradiation, with a corresponding effluent temperature of $30 \pm 1\,^\circ\text{C}$ for both peaks (concentration: 1.0 g/L; injection volume: 20 μL; microwave power output: 30 W; mobile phase: 70:30 (methanol/water); flow rate: 1.2 mL/min).

The chromatographic pulses were all obtained under linear conditions at a mobile phase composition of 70% aqueous solution of methanol, mobile phase flow rate of 1.2 mL/min, and at the same experimental conditions and temperatures. Qualitative comparison of the chromatograms in Figs. 1–4 shows sharper peaks for the band profiles obtained with microwave irradiation than without irradiation. The enhancement of the peak profiles suggests an increase in the intraparticle diffusivity of the solute inside the pores, as a result of the dielectric polarization of the microwave-active molecules in the mobile phase. This sharpening of the peak due to the increase in the mobile phase diffusivity by microwave irradiation was also previously reported [12].
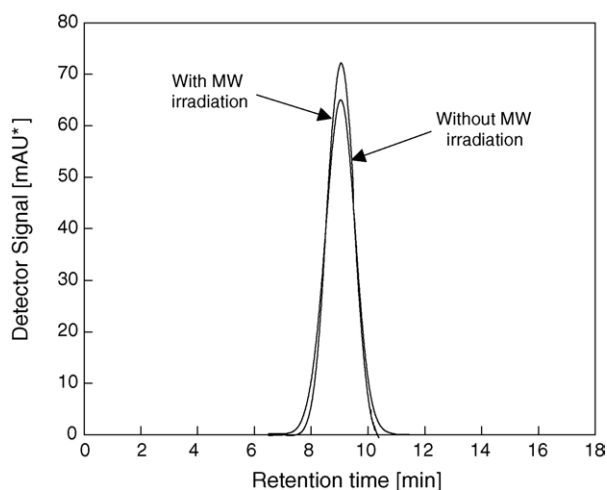

Fig. 4. Comparison between the experimental band profiles of propylbenzene obtained with and without microwave irradiation, with a corresponding effluent temperature of $30 \pm 1\,^\circ\text{C}$ for both peaks (concentration: 1.5 g/L; injection volume: 20 μL; microwave power output: 30 W; mobile phase: 70:30 (methanol/water); flow rate: 1.2 mL/min).

The chromatographic peaks obtained experimentally were analyzed using the conventional method of moments [21–25]. Long ago, this method was proven to be the most effective in the accurate determination of the properties of chromatographic peaks [26–27]. It has now become the most conventional method for this kind of investigations [21]. More details on moment analysis can be found elsewhere [28–32]. Information on the thermodynamics of equilibrium between the mobile and the stationary phases, and on the mass transfer kinetics between the two phases in the column are derived from the first absolute moment (μ_1) and the second central moment (μ_2') of the bands, respectively, defined as follows:

$$\mu_1 = \frac{\int C(t)t \, \mathrm{d}t}{\int C(t) \, \mathrm{d}t} \tag{1}$$

$$\mu_2' = \frac{\int C(t)(t - \mu_1)^2 \, \mathrm{d}t}{\int C(t) \, \mathrm{d}t} \tag{2}$$

where $C(t)$ is the chromatographic band profile. A quantitative estimate of the influence of MW irradiation on the column performance was obtained by determining the number of theoretical plates, N, of the column. This number was calculated using the relationship between the first and the second moments as stated in the following equation:

$$N = \frac{\mu_1^2}{\mu_2'} \tag{3}$$

Table 1 compares the values calculated for the plate numbers of the column under MW irradiation at 15 and 30 W power outputs and of the non-irradiated column as shown in Figs. 1–4. The values obtained are ca. 18% higher with irradiation under a power of 30 W than without irradiation with a corresponding effluent temperature of $30 \pm 1\,^\circ\text{C}$. This increase in the plate number is similar to that reported earlier [12]. Plate numbers of 69 and 31% larger under MW irradiation than without it were observed for 2-naphthol and benzene, respectively. The larger increase reported is explained by the larger MW power applied that corresponded to a steady outlet temperature of 75 °C. In our work, the measurements were made with a lesser power output. This suggests that, increasing the MW power output could increase considerably the plate number of the column.

Fig. 5 shows the temperature profiles of the mobile phase stream at the column outlet, for four different MW power outputs and two mobile phase compositions. At 70% aqueous solution of methanol, the effluent temperatures at 15 and 30 W power outputs are 25 ± 1 and $30 \pm 1\,^\circ\text{C}$, respectively,

Table 1
Calculated number of theoretical plates as a function of microwave power output

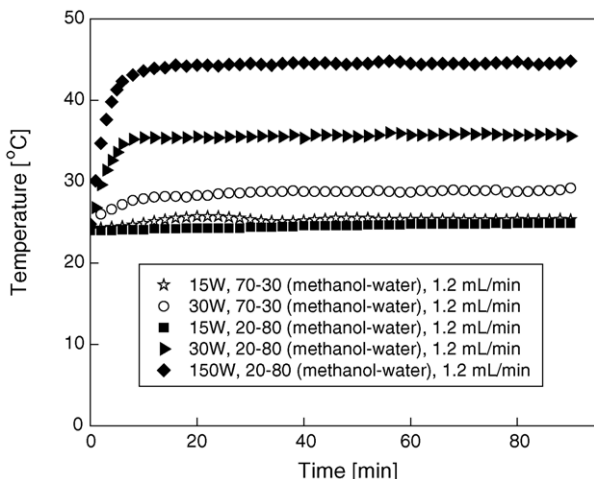| C_0 (g/L)	Number of theoretical plates (N)			
	15 W-25 °C	0 W-25 °C	30 W-30 °C	0 W-30 °C
1.0	347	326	354	300
1.5	347	327	354	303

Fig. 5. Temperature profiles measured at the column outlet at 15, 30, and 150-W microwave power outputs, 70:30 and 20:80 (methanol/water) mobile phase compositions, and 1.2 mL/min mobile phase flow rate.

Fig. 7. Comparison between the experimental breakthrough-elution curves of phenol obtained with and without microwave irradiation (concentration: 10–50 g/L; injection volume: 5 mL; microwave power output: 50 W; mobile phase: 20:80 (methanol/water); flow rate: 1.2 mL/min).

while the effluent temperatures at 20% aqueous solution of methanol at 15, 50, and 150 W are 25 ± 1, 35 ± 1, and $45 \pm 1°C$, respectively. This shows that the heating is negligible at 15 W and that it increases much with increasing MW power. The radial temperature gradient becomes significant above ca. 30 W, which probably affects negatively the column efficiency.

3.2. Comparison of chromatograms obtained under overloaded conditions

The effect of MW irradiation on the thermodynamics of equilibrium between the mobile and stationary phases was studied by comparing qualitatively the breakthrough curves of phenol. Figs. 6–8 show the curves obtained for phenol dissolved at 10, 20, and 50 g/L, respectively, in an aqueous
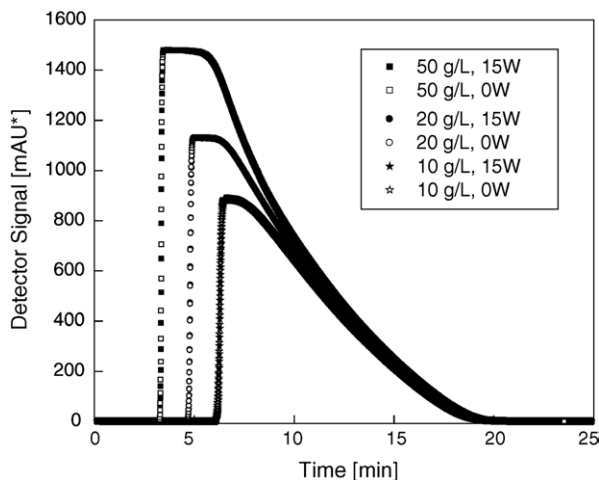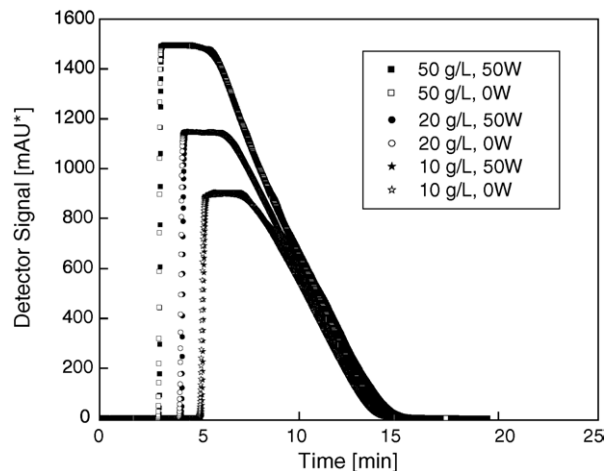
solution of methanol (20%), at a flow rate of 1.2 mL/min, and with microwave power outputs of 15, 50, and 150 W, respectively. The temperature profiles measured at the column outlet at these MW power outputs are shown in Fig. 5. In the whole range of concentrations considered, there is no significant difference in the profiles or elution times between the breakthrough curves obtained with and without MW irradiation, under the same experimental conditions and at the same temperatures. The perfect matching of the breakthrough curves of phenol obtained with and without irradiation, under all conditions suggests that the thermodynamics of equilibrium between the mobile and the stationary phases is not affected by the application of microwave energy. This result may be explained by the transparency of silica-based stationary phases to microwave irradiation at 2.45 GHz. The
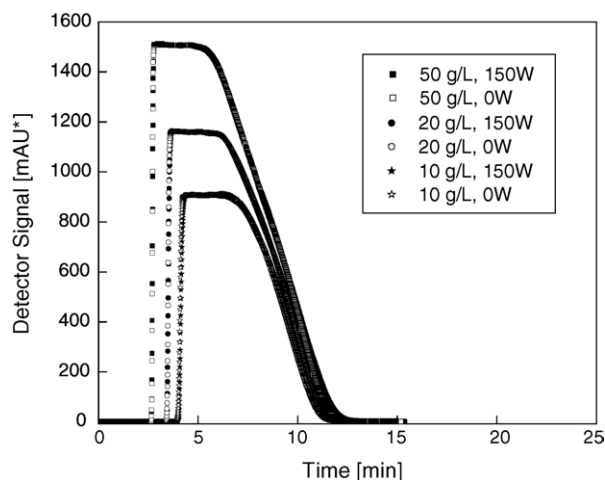

Fig. 6. Comparison between the experimental breakthrough-elution curves of phenol obtained with and without microwave irradiation (concentration: 10–50 g/L; injection volume: 5 mL; microwave power output: 15 W; mobile phase: 20:80 (methanol/water); flow rate: 1.2 mL/min).

Fig. 8. Comparison between the experimental breakthrough-elution curves of phenol obtained with and without microwave irradiation (concentration: 10–50 g/L; injection volume: 5 mL; microwave power output: 150 W; mobile phase: 20:80 (methanol/water); flow rate: 1.2 mL/min).

pronounced tailing that can be observed in the diffuse rear boundaries of the breakthrough curves is a consequence of using a packing material that is made of particles having a large diameter. This choice was made purposely in order to magnify the mass transfer resistances in the stationary phase.

4. Conclusion

Microwave irradiation has a significant effect on the mass transfer kinetics and practically no effect on the thermodynamics of equilibrium in an RPLC column. The first conclusion results from the sharper peaks obtained for propylbenzene under linear conditions, when the column is subject to MW irradiation. The column plate number increased by ca. 18% when the column was irradiated at a 30 W power output. The second conclusion arises from the lack of significant differences between the profiles and elution times of the high concentration breakthrough profiles of phenol obtained with and without MW irradiation. The close similarity between the rear diffuse boundaries of the breakthrough profiles is not inconsistent with a slightly higher column efficiency under MW irradiation as these profiles are not very sensitive to slight changes in column efficiency.

Acknowledgements

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