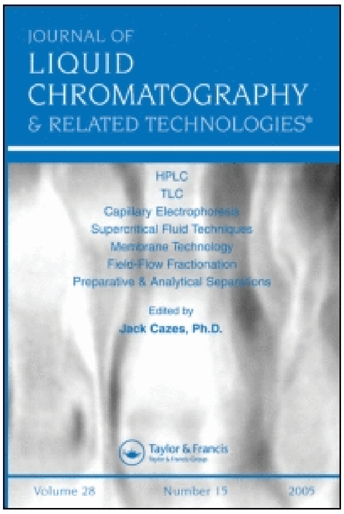

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COMPARISON OF TWO NOVEL TECHNIQUES FOR ELIMINATING LIQUID CHROMATOGRAPHY GRADIENT GHOST PEAKS

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INTRODUCTION

Distilled water to organic gradients in reversed phase liquid chromatography are often plagued with UV-detectable "ghost" peaks that can obscure sample peaks and complicate interpretation of results.[3,4] These contaminants usually come from the distilled water[3] although it is possible for them to originate in the organic eluent.[4] The ghost-peak problem can be eliminated by using specially prepared "LC-grade" water, although this is expensive. In addition water can be cleaned with the Milli-Q ion-exchange system, containing a carbon absorption column[5] but not where these systems contain buffers or organic amine eluting agents such as triethylamine phosphate. Gurkin and Ripphahn have shown that a C-8 silica column can be used off-line in a low-pressure mode to clean water.[6]

A very effective on-line clean-up system that can be used with buffered or amine containing eluents was recently described.[4]

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This involves inserting a large particle "porous polymer" styrene-divinylbenzene column in the high pressure aqueous line before the gradient mixer. This "eluent conditioner column" removes ghost peaks contributed not only by the water but also from such buffer additives as triethylamine, phosphoric acid, sodium hydroxide, boric acid and ammonium hydroxide.

Several publications have shown that UV irradiation of batches of distilled water also eliminates UV detectable ghost-peaks.[7,8] Two batch UV irradiators are now commercially available (Barnstead, Boston, MA, U.S.A. and Photronix, Medway, MA, U.S.A.).

This work shows that a flow-through, on-line UV irradiator may also be used to eliminate water-derived ghost peaks with the advantage that no water transfers are needed and the usual 1/2 to 3 hour wait[4] is not required. This on-line UV irradiator is shown here to be as effective for eliminating water derived ghost-peaks as the aqueous eluent conditioner column if water only is used as an eluent.

The flow through irradiator described herein is made by using Teflon tubing wrapped around the UV lamp in a batch irradiator for the water flow line. Scholten et. al.[9] showed earlier that Teflon is transparent to UV light.

Low wavelength 189 nm UV irradiation of water is believed to eliminate ghost peaks by photo-oxidizing trace level contaminating organic compounds. The principle of photo-oxidation is well established in organic chemistry[10] and in areas such as the LC halide photo-conductivity detector[11] and in the organic carbon analyzer[12] for environmental analysis.

EXPERIMENTAL SECTION

Materials and Methods

Figure 1 shows the modified Photronix (Medway, MA, USA) Model 816 HPLC batch UV irradiator. The flow-through section was constructed by wrapping Teflon tubing (0.15 cm I.D. x 0.30 cm O.D. part 200-32, Rainin Instrument Co., Woburn, MA, USA) in 33 coils (205 cm total length) around the 35 watt UV lamp used in the Photronix reservoir. The ends of the Teflon tubing were tied in place with wire. The hold-up volume is ca. 3.6 mL. The lamp with the coiled tubing was then inserted back into the protective quartz test tube sealed to the top of the Photronix reservoir. Further the final chamber can be used to batch clean water for buffer preparation for one LC and simultaneously operate as a flow-through irradiator for a second LC. Additionally, water in the batch irradiator acts as a coolant for the flowing water system.

A Hewlett-Packard (Avondale, PA) 1084D liquid chromatograph was used and at least four replicate runs were obtained for each gas sparging or lamp on-off condition. Cylinder gas bubbled through a frit, was used for each condition of oxygen saturation, air saturation, or helium sparging. Distilled water (Belmont Springs, Belmont, MA, USA) was from 5 gallon polyethylene bags in cardboard boxes. Acetonitrile was HPLC grade (Burdick and Jackson, Muskegon, MI, USA).

For work using the porous polymer, this column was installed in the aqueous eluent line just after the pump and before the gradient mixer. This 25 x 0.46 cm column was dry tap-packed with 150-200 um Chromosorb 101 styrenedivinylbenzene porous polymer (Supelco, Bellefonte, PA). Each morning this column was cleaned

FIGURE 1. Photronix HPLC reservoir with the lid raised to show the Teflon tubing for the flow-through UV irradiator coiled around the lamp and running to an auxilliary flask of distilled water.

by turning a valve arrangement so that ca. 15-25 mL of acetonitrile flushed the aqueous line. A dozen flush cycles between aqueous and acetonitrile may be required for initial cleaning.

RESULTS AND DISCUSSION

Figures 2 and 3 show that a flow-through irradiator having a water residence time of only 0.9 min works very well. Figure 2 shows that the 254 nm detectable ghost peaks (lower row) are removed when the flow-through irradiator is turned on (upper row). This is true for flows as high as 4 ml/min for water saturated with oxygen (chromatogram b), water saturated with air (chromatogram d) and for water "degassed" of nitrogen and oxygen by sparging with helium (chromatogram f). For helium sparged water passed through the on-line UV irradiator, the disappearance of peaks found with 254 nm UV detection was also shown with detection at 210 nm (Figure 3, chromatogram a, lamp off. vs. chromatogram b, lamp on). Since levels of dissolved oxygen should be low in this case, the mechanism of "photo-oxidation" may be more complicated than the simple reaction of major levels of oxygen with the organic impurities under the influence of UV light. Also with 210 nm detection, the ghost peaks were shown to disappear using the flow-through UV irradiator with air saturated water (chromatogram c, lamp off vs. chromatogram d, lamp on) but in this case the baseline shift between 4% and 96% acetonitrile was less than with helium degassing. Part of this better match between the initial 4% acetonitrile baseline and the final 96% acetonitrile baseline

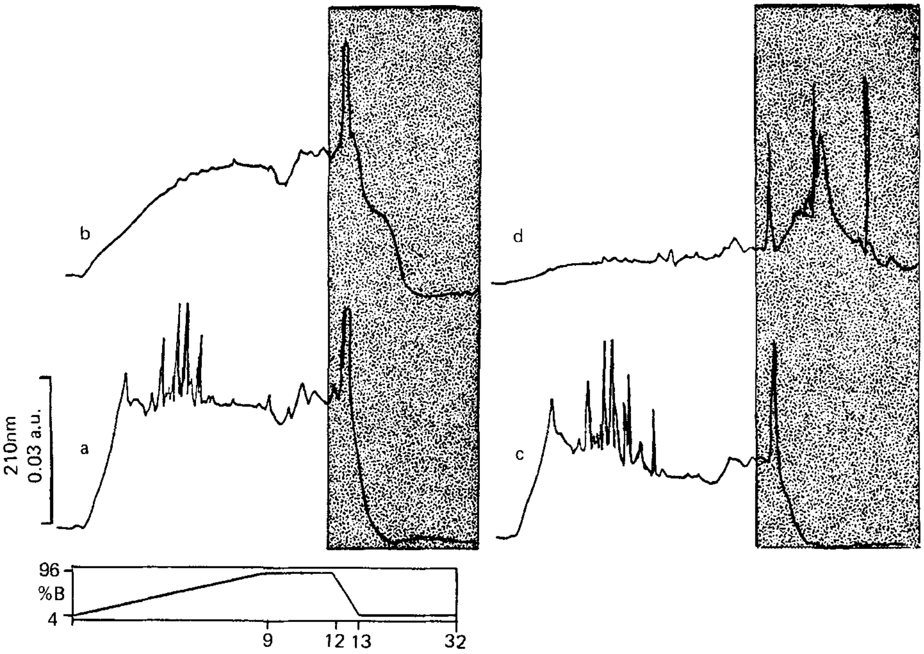

FIGURE 2. Gradient liquid chromatograms showing that 254 nm detectable ghost peaks from commercial distilled water (lower row) are eliminated with the Teflon flow-through irradiator (upper row) for water saturated with oxygen (left), water saturated with air (middle), and water degassed with helium (right). Flows of 4 ml/min are used with a reversed phase Waters C-18 Radial Comparession column (10 x 0.5 cm), 10 micron particles. The gradients are from 0 to 96% acetonitrile using the gradient shape shown at the bottom with initial isocratic water for 19 min (76 ml). The hatched portion is on the return of the gradient.

comes from the fact that turning on the irradiator causes the 4% acetonitrile baseline to shift up by 0.009 absorbance when air saturated vs. a shift up to 0.005 absorbance when helium degassed. The cause of these shifts was not investigated.

In runs similar to those shown in Figure 2 (using 4 mL/min), but using 2 mL/min and 1 mL/min (irradiated residence times of 1.8 and 3.6 min, respectively), the baseline showed nearly complete

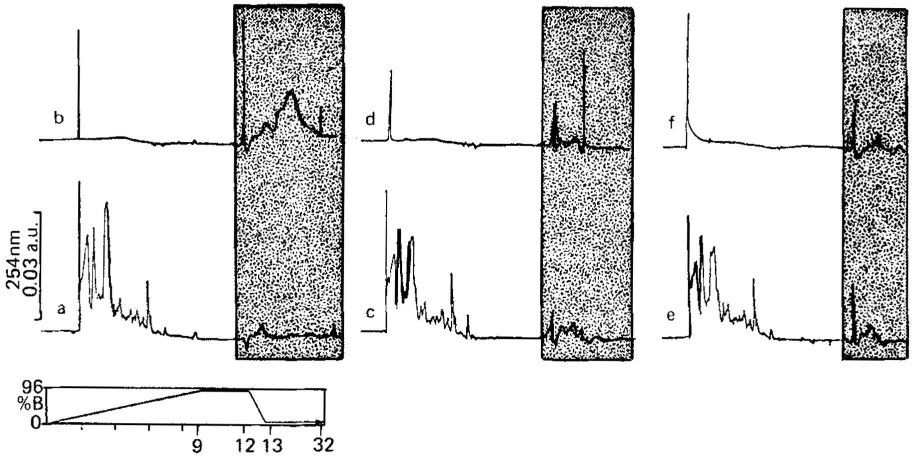

FIGURE 3. Gradient liquid chromatograms showing that 210 nm detectable ghost peaks from commercial distilled water (lower row) are eliminated with a Teflon flow-through irradiator (upper row) for water degassed with helium (left) and water saturated with air (right). Conditions are as in Figure 2 except that the gradient is from 4 to 96% acetonitrile and detection is at 210 nm.

absence of ghost peaks for all three conditions of gas purging (oxygen, air, and helium). Thus it appears that major levels of oxygen are not necessary to "photo-oxidize" the contaminants in water.

One complication was found with air-saturated water when using slow 1 mL/min flows with the on-line UV irradiator. Gas that bubbled out of solution as the water warmed in the irradiator caused occasional flow irregularities. Bubble formation was not observed with the helium-degassed water. Thus helium-degassed water allows slow flows through the irradiator with no flow irregularities.

It should also be stressed that UV irradiation is not useful with water containing phosphates or organic additives. Potassium

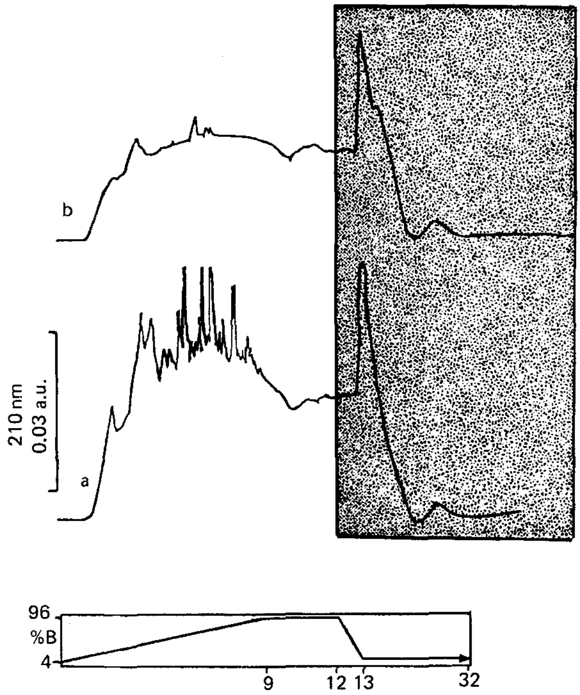

FIGURE 4. Gradient liquid chromatograms (4 to 96% acetonitrile) showing that 210 nm detectable ghost peaks from commercial distilled water (a) are mostly eliminated with a porous polymer (Chromosorb 101) "eluent conditioner column" in the aqueous line (b). Conditions are as in Figure 2 except that a 3 cm Brownlee C 18 guard column and a 15 cm Zorbax C 18 column are used at 3 ml/min and no UV irradiator is used.

phosphate buffer decomposes on UV irradiation forming highly UV-absorbing species that "break-through" the C-18 column after many gradient cycles.

When a porous polymer eluent conditioner column was placed in the aqueous eluent line after the pump (Figure 4, chromatogram b), the 210 nm ghost-peak level was found to be similar to that found with helium degassed water (Figure 3, chromatogram b). The disadvantage of this porous polymer eluent conditioner column is that it requires re-conditioning about every 2 days with a 5 min acetonitrile wash. However, it does remove most ghost peaks contributed by buffer or amine additives put in the water.

This work shows that for liquid chromatography using water only as one reversed-phase gradient component, ghost peaks can be eliminated by simply continuously degassing the water with helium and running the water through the flow-through on-line UV irradiator described here. Showing similar effectiveness is the porous polymer eluent conditioner column which in addition removes most ghost peaks contributed by buffer or amine additives.

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