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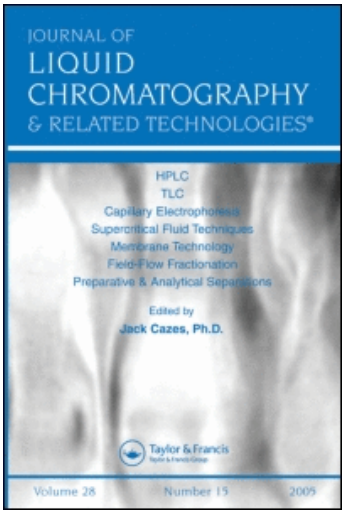

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A Comparison Between a Micro and an Ultramicro Circle® Cell for on-Line Ft-Ir Detection in a Reverse Phase HPLC System

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A COMPARISON BETWEEN A MICRO AND AN ULTRAMICRO CIRCLE® CELL FOR ON-LINE FT-IR DETECTION IN A REVERSE PHASE HPLC SYSTEM

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ABSTRACT

Caffeine and theophylline peaks separated on a 300 x 7.8 mm C_{18} column could be distinguished qualitatively by FT-IR detection using two types of cylindrical internal reflectance (CIRCLE®) cells. Detection limits and limits of identification, on the order of 0.2%, are lower for the micro CIRCLE® cell as compared to the ultramicro CIRCLE® cell. Maximum sample capacity of the column used was 50uL of a 0.4% solution of caffeine(1.0 micromoles).

INTRODUCTION

On-line infrared detection using a flow cell for reverse phase HPLC is not a well established technique. Generally this

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is because the pathlength requirement with aqueous solvents presents two severe limitations to reverse phase IR detection. First, even at pathlengths of less than 25um, aqueous solvents give rise to large background signals.[1] Secondly, such short pathlengths imply that large amounts of materials must be injected into the HPLC system. [2] To the best of our knowledge no previously published studies have addressed the question of sample capacity or column overloading specifically with FT-IR detection.

The CIRCLE (cylindrical internal reflectance) cell is an attenuated total reflectance accessory which has an effective infrared pathlength sufficiently short (<10um) to make it amenable to aqueous samples.The successful application of the micro CIRCLE cell and the ultra-micro CIRCLE cell to aqueous flow injection analysis (FIA) with FTIR detection has prompted further investigations of these detection systems. [3-5] We now wish to report the use of both cells for FTIR detection reverse phase HPLC. Sabo et. al [6] demonstrated the feasibility of using a CIRCLE cell for HPLC. However, the mixture separated (acetophenone, nitrobenzene, and ethylbenzoate) could have been qualitatively identified by a diode array UV detector. In addition, data for only one concentration mixture was reported, and this represented an injection of 2.0mg each of acetophenone and ethylbenzoate and 1.0mg of nitrobenzene. Detection limits were not determined for any of the compounds. Redmond et. al [7] utilized a micro CIRCLE cell in a study which demonstrated new data analysis methods for suboptimal separations. Kalman filtering was applied to enhance weak

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infrared signals in cases where there was significant overlap of peaks in the chromatogram.

Analyte identification, not detection limit, is the anticipated main strength of HPLC-IR because closely related compounds can be identified by their infrared spectra. This study investigates the potential for using a micro CIRCLE cell for the identification of two compounds differing only by a methyl group, caffeine and theophylline, after separation by HPLC. Detection limits are reported for these compounds using both types of CIRCLE cells.In addition, the sample capacity range recommended for HPLC with CIRCLE cell detection is given.

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Experimental

Theophylline and caffeine were used as received without further purification. The mobile phase consisted of spectral grade methanol and triply distilled water, 60/40, degassed with helium. Samples were prepared in the following manner: 0.5499g theophylline and 1.0927g caffeine were dissolved in 100ml of 40/60 methanol/water to give a solution 0.5% (5PPT) in theophylline and 1.0% (10PPT) in caffeine. This solution was used as a stock to prepare mixed standards ranging from 0.05% (.5PPT) theophylline and 0.1% (1.0PPT) caffeine to 0.5% (5PPT) theophylline and 1.0% (10PPT) caffeine. Standards for flow injection analysis (FIA) were prepared from a 10PPT stock solution of caffeine.

In both CIRCLE cells, the internal reflection element (IRE) is a cylindrical ZnSe rod held in place by Teflon o-rings. The solution to be analyzed bathes the exposed portions

of the ZnSe rod. The IRE of the micro CIRCLE cell is 4.0 cm X 0.3 cm, and the cell has an internal volume of 24uL, while the ultra micro cell has an IRE with dimensions of 1.7 X 0.13cm and an internal volume of 1.75uL. Briefly, the micro CIRCLE cell [8] has an average acceptance angle of approximately 50° , while the ultramicro cell [5] has an average acceptance angle of approximately 56 . These differences in the acceptance angle, along with the differences in the dimensions of the IRE lead to an average number of reflections of 7.5 for the micro cell and 4.9 for the ultra-micro cell. Multiplying the average number of reflections times the depth of penetration gives effective "pathlengths" of 4.65×10^{-4} and 3.18×10^{-4}cm, respectively, for the micro and ultra-micro cells.

The HPLC system consisted of a Beckman Model 110A pump, a Rheodyne Model 7010 air actuated injection valve with a 50uL loop, and an Altex Model 153 analytical UV detector equipped with a 254nm filter. The column used was a Waters C_{18} u-Bondapak 7.8mm x 30.0 cm reverse-phase column. Ultra-violet chromatograms were recorded on a Fisher Recordall (Fisher Scientific) stripchart recorder. Figure 1 is a block diagram of the experimental arrangement showing the connection of the detectors in series.

The infrared spectra were recorded with a Perkin-Elmer Model 1800 FT-IR controlled by a Perkin-Elmer Model 7500 Professional computer. The front sample compartment of the spectrometer was equipped with a Spectra Tech IR-PLAN infrared microscope and it's narrow band MCT detector ($.025^2$ cm, Specific detectivity (D^*)= 6.79×10^{10} cm $Hz^{1/2}$ w^{-1}). The micro

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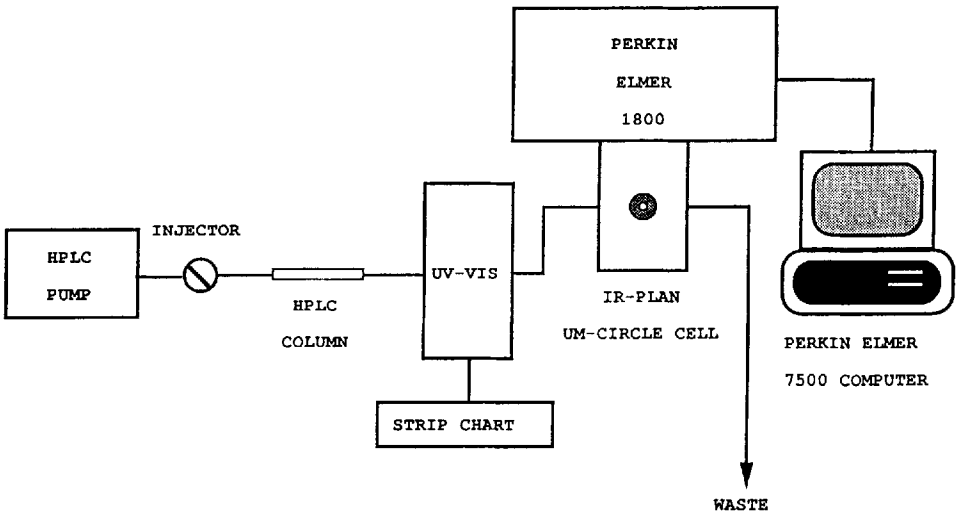

1. Diagram of the experimental set up.

CIRCLE cell was used in the rear sample chamber of the FT-IR with the instrument's narrow band MCT detector, (D^*=5.78x10^{10} cm $Hz^{1/2}$ w^{-1}) while the ultra-micro cell was used in conjunction with the Spectra Tech IR-PLAN microscope. The FT-IR was operated at 8cm^{-1} resolution with a weak (Norton-Beer) apodization function. Cassegrainian mirrors mounted on both ends of the micro cell serve to condense and expand the infrared beam. The Cassegrainian objective and condenser of the IR-PLAN infrared microscope serve the same purpose when the ultra-micro CIRCLE cell is placed upon the sample stage.

Infrared spectra were obtained on-the-fly by two methods. A program was written to obtain five coadded infrared scans in the 2000-1100cm^{-1} region, with the help of the Obey programming capabilities of the Perkin-Elmer 7500 Professional

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computer. The data collection was initiated manually when the peak on the UV chromatogram reached a predetermined height. The height was determined by chromatographing a standard, without collecting any infrared data, and determining the peak height which corresponded to a 1mM peak width (24s) for theophylline, or a 2mM peak width (48s) for caffeine. Because of severe column overloading above concentrations of 0.75%, the caffeine peak did not always narrow to a peak width of 1mm. Above 0.75% caffeine, the peak was broad and flat at its maximum. For this reason, and for the sake of consistency, a peak width of 2mm was chosen for all caffeine injections.

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The second method for acquiring spectra utilized the GC-IR software installed on the Perkin-Elmer 7500 computer. This software allows for the collection of approximately 2 scans per second, which are stored sequentially as files. A chromatogram can then be reconstructed from the files based upon total infrared activity (Gram-Schmidt) or on the infrared activity in specific spectral regions. For this study the ranges between 1700-1600cm^{-1} and 1400-1200cm^{-1} were used to reconstruct the chromatograms. After reconstruction of the chromatograms, five scans corresponding to a peak maximum were coadded.

In the FIA portion of the study, the column and UV-Vis detector were removed and caffeine solutions were injected into a carrier stream of triply distilled, helium degassed, water. The caffeine concentrations in the FIA experiments ranged from 0.2% to 1.0% w/v. The FIA experiments were run using both the micro and ultra-micro flow cells. Data were

collected with the use of the Perkin-Elmer GC-IR software, and five scans corresponding to the concentration maximum were coadded.

To check for column overloading, and determine sample capacity, peak widths for caffeine were measured from the UV chromatagrams.

Results and Discussion

Figure 2 shows the change in peak width vs. the concentration of caffeine injected. The intercept of the line represents the peak width at infinite dilution. As a rule of thumb, the concentration where the peak width has become larger by 10% than the intercept represents the maximum sample capacity of the column. This capacity was determined to be approximately 0.4%. Injecting higher concentrations may result

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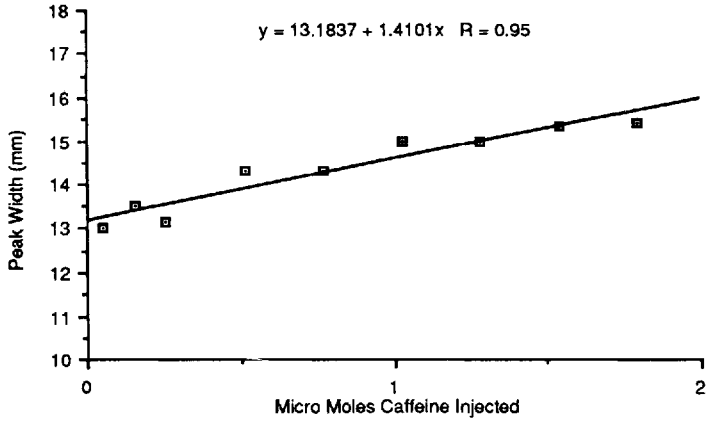
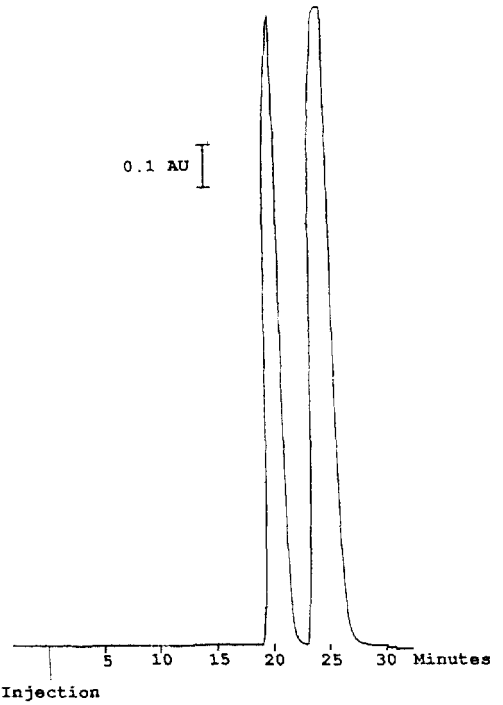


2. Peak width (mm) vs. micromoles caffeine injected.



3. UV chromatogram of caffeine (0.8%) and theophylline (0.4%)

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in severe band broadening or shifts in retention time. At concentrations above 0.8% the caffeine peak became flattened (Figure 3) which also indicates column overloading. Since it has been demonstrated that spectroscopic data acquired with the CIRCLE cell can be linear up to injection sizes of 20mg[3] in an FIA experiment, in an HPLC experiment the column will likely become overloaded before exceeding the capabilities of the detection system. High capacity columns with a diameter of at least 7.0mm are a necessity wnen doing on-line infrared

detection with the CIRCLE cell. Columns of standard dimensions (25cm X 4.6mm) will limit the samples that can be examined by HPLC with a CIRCLE cell and infrared detection.

Since caffeine and theophylline only differ by a methyl group, the UV-Vis spectra are nearly identical making qualitative identification difficult. Figures 4A and 4B show the IR spectra for a solution containing 1.0% caffeine and 0.5% theophylline, respectively, acquired using the micro CIRCLE cell. The carbonyl absorption bands for caffeine occur at 1641cm^{-1} and 1700cm^{-1} and at 1645cm^{-1} and 1701cm^{-1} for theophylline. A pyrimidine ring vibration[9] occurs at 1554cm^{-1} and 1562cm^{-1} for caffeine and theophylline, respectively. To distinguish the spectra, an imidazole ring vibration[9] at 1241cm^{-1} is an excellent marker for caffeine.

Figure 5 shows the spectrum of a 0.1% injected solution of caffeine. It is clear that a carbonyl group is present in the sample, but no other information can be gleaned from the spectrum. The band at 1554cm^{-1} is becoming visible at a concentration of 0.2%, but Figure 6A probably represents the minimum identifiable quantity for caffeine (0.3%). Figure 6B is a spectrum of the minimum identifiable quantity for theophylline, 0.15% (1.5PPT). The difference in limits of detection between caffeine and theophylline is due to the greater intensity of the pyrimidine ring band of theophylline at 1562cm^{-1} than the corresponding band of caffeine at 1554cm^{-1}. In addition, the concentration in the CIRCLE cell at the time the spectra are acquired is slightly higher for theophylline than for caffeine because the spectra are

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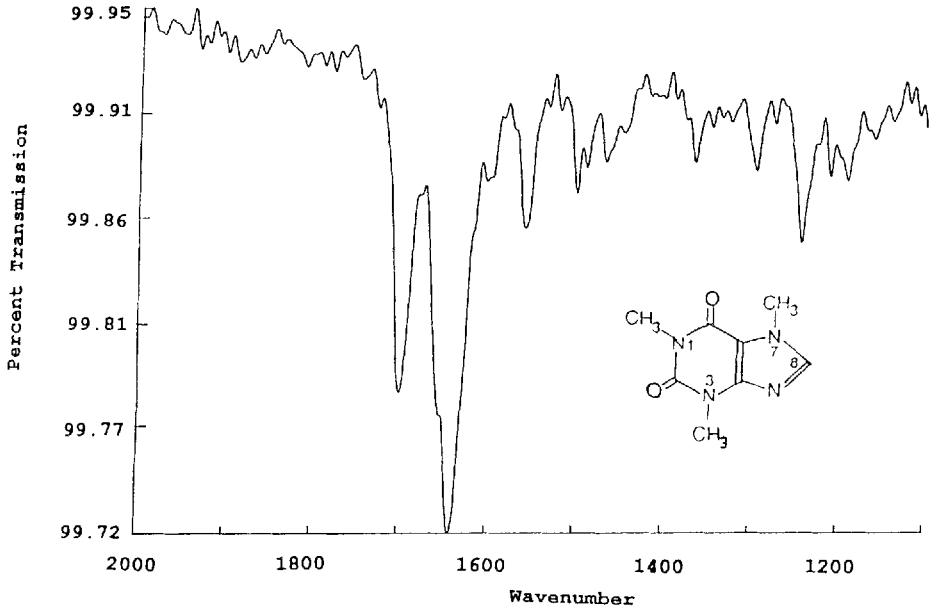
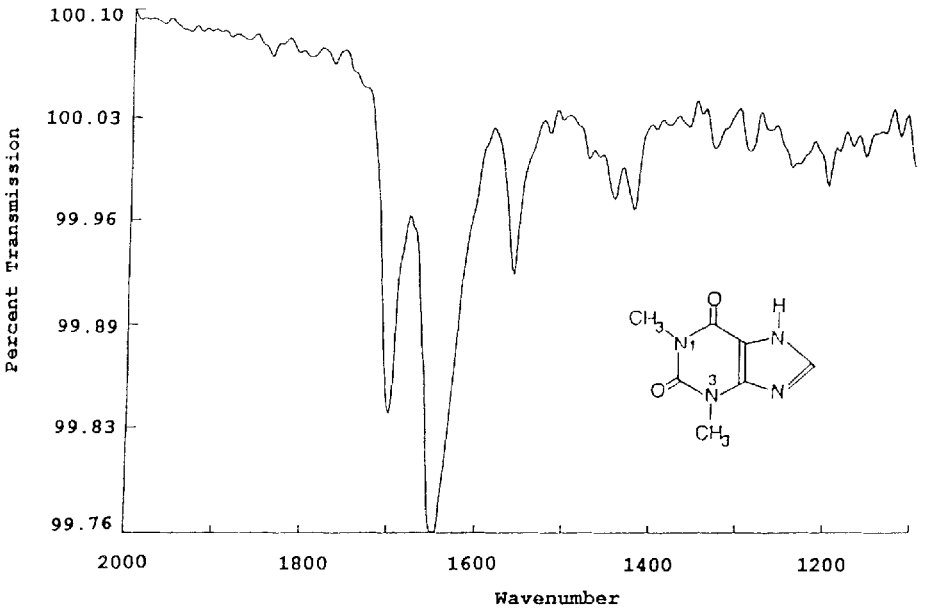


4A. Spectrum of a 1.0% caffeine solution.



4B. Spectrum of a 0.5% theophylline solution.

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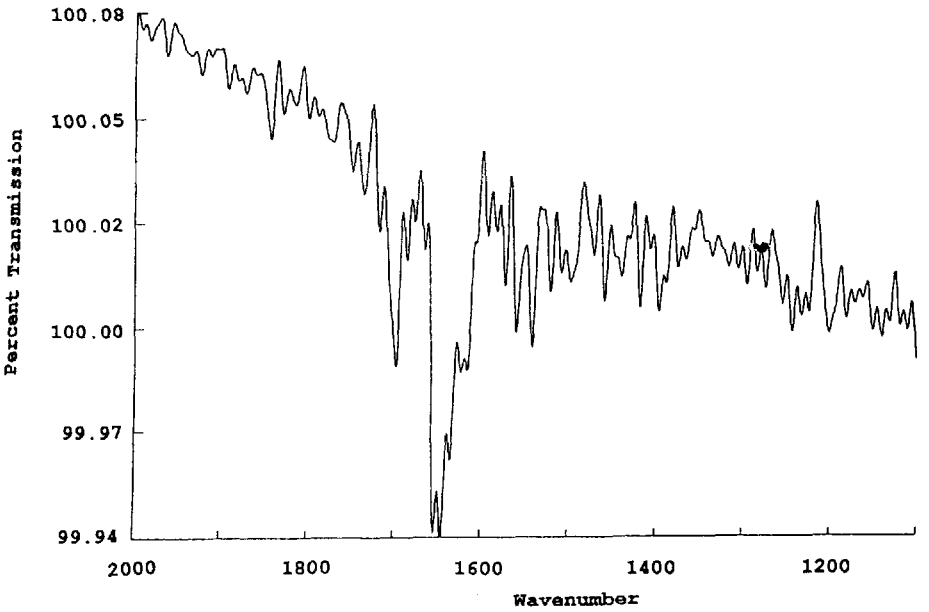

5. Spectrum of a 0.1% solution of caffeine.

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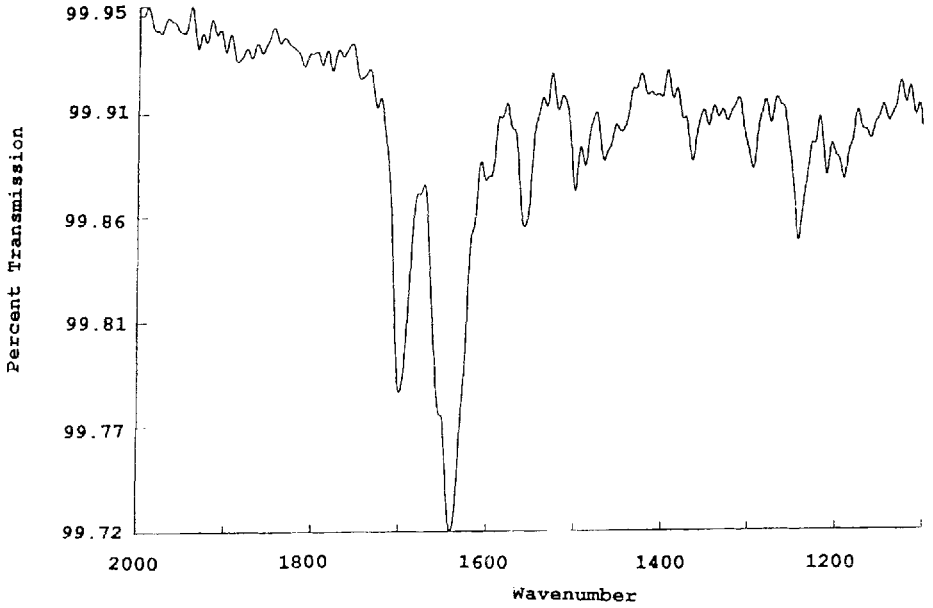
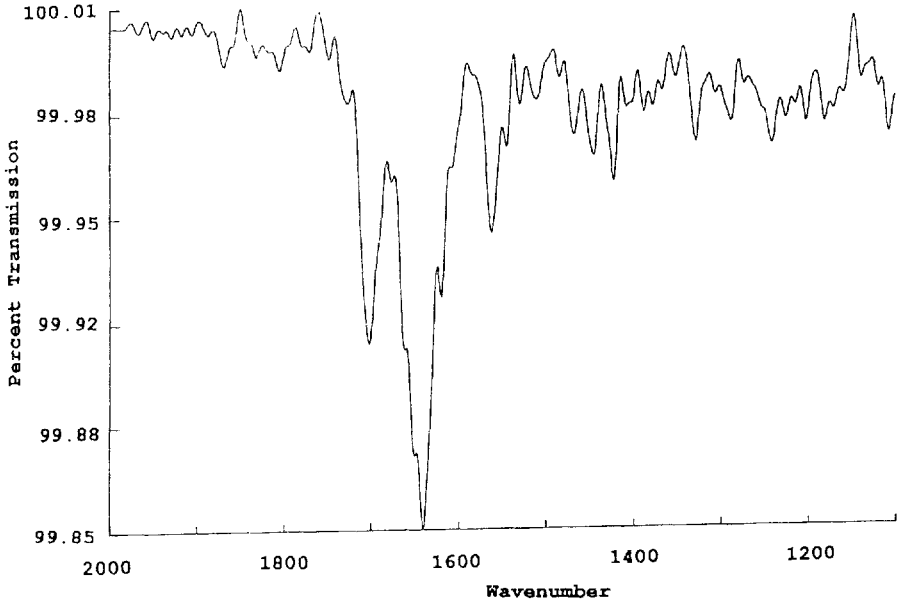
acquired closer to the apex of the chromatographic peak. The previous spectra were all obtained on-the fly with an acquisition program written with Perkin Elmer's Obey programming capabilities. Identical spectra were obtained with the Perkin Elmer GC-IR software.

The same set of HPLC experiments was then performed with the ultra-micro CIRCLE cell. Figures 7A and 7B show the spectra for 1.0% caffeine and 0.5% theophylline, respectively, acquired on-the-fly. For the caffeine spectrum, there appears to be some carbonyl absorption. In the theophylline spectrum there is increased noise in the carbonyl region, but no



6A. Spectrum of a 0.3% solution of caffeine.



6B. Spectrum of a 0.15% solution of theophylline.

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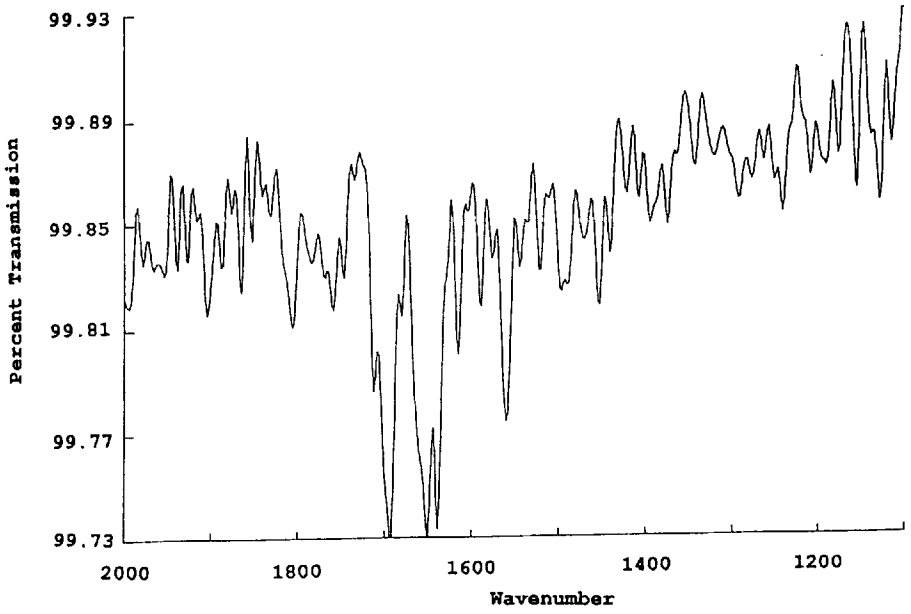
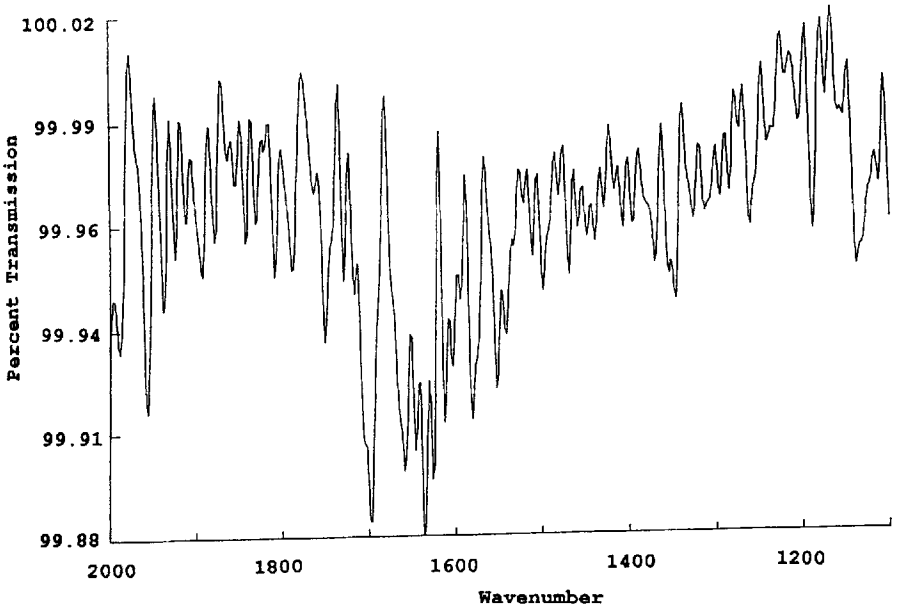


7A. Spectrum of a 1.0% solution of caffeine acquired with the ultra micro CIRCLE cell.



7B. Spectrum of a 0.5% solution of theophylline acquired with the ultra micro CIRCLE cell.

Table 1: Limits of Detection and Identification for Caffeine

Experiment	LOD*	LID*
uM HPLC	1.0%(0.5mg)	>>1.0%(0.5mg)
M HPLC	0.2%(0.1mg)	0.3%(0.15mg)
uM FIA	0.5%(0.25mg)	>1.0%(0.5mg)
M FIA	<0.2%(0.1mg)	0.2%(0.1mg)

uM- Ultra micro CIRCLE cell
M- Micro CIRCLE cell

*- Limit of Detection (LOD) is defined as the minimum concentration which produces a carbonyl absorption of greater than three (3) times the background noise level. Limit of Identificaton (LID) is defined as the minimum concentration which produces an absorption, other than a carbonyl band, of greater than three (3) times the background noise level.

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obvious infrared absorption. Identical spectra were obtained when the GC-IR software was utilized for data collection.

The tremendous disparity in detection limit between the two cells prompted an FIA investigation using caffeine as the analyte. To insure that the spectra corresponded to the maximum analyte concentration, the GC-IR software was used for data acquisition. In Table 1 it is seen that the limit of detection (LOD) and the limit of identification (LID) for caffeine are always lower when using the micro cell as opposed to the ultramicro cell. This data trend is not unexpected in light of previous work [5]. The LOD and LID for caffeine are

also always lower when doing FIA experiments instead of HPLC experiments, due to the lower dispersion (less band broadening) in the FIA experiments. These data show detection limits in the sub-mg range are possible. The shorter pathlength , increased background due to the proportionally larger Teflon o-rings, and lower energy throughput of the microscope system contribute to the generally poorer detection limits of the ultra-micro cell as compared to the micro cell.[5]

Conclusions

The sample capacity of 0.4% for the HPLC column is exceeded when using the ultra micro CIRCLE cell but is satisfactory when using the micro CIRCLE cell. Because of high background absorbance and low S/N, the amount of material injected must be quite large, from a chromatographic standpoint, in order to generate a useful spectrum from either cell. The ultra-micro cell is useful for flowing or static measurements of small volume solutions where the component(s) of interest are present in concentrations of several percent. While detection limits achieved with the micro CIRCLE cell do not rival the limits achieved by other methods [10-12] for the same chromatographic system, the limits of identification represent a realistic quantity of injected material for some applications. A large capacity column is important for reliable chromatography. The 30.0cm X 7.8mm column used in this study is adequate for HPLC with micro CIRCLE cell detection. The micro CIRCLE cell is a relatively inexpensive

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and simple accessory for obtaining infrared spectra in flowing systems to assist in qualitative identification.

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References

1. P. R. Griffiths, S. L. Pentoney Jr., A. Giorgetti, and K. H. Shafer, **Anal. Chem.** 58(13), 1349A-66A (1986)

2. J. W. Hellgeth and L. T. Taylor **J. Chromatogr. Sci.** 24(11), 519-28 (1986)

3. B. E. Miller, N. D. Danielson, and J. E. Katon **Appl. Spectrosc.** 42(3), 401-05 (1988)

4. D. K. Morgan, N. D. Danielson, and J. E. Katon **Anal. Lett.** 18(A16), 1979-98 (1985)

5. P. T. McKittrick, N. D. Danielson, and J. E. Katon **Submitted, The Microchemical Journal**

6. M. Sabo, J. Gross, J. S. Wang, and I. E. Rosenburg **Anal. Chem.** 57(9), 1822-26 (1985)

7. M. Redmond, S. D. Brown, and H. R. Wilk **Anal. Lett.** 22(4), 963-79 (1989)

8. R. P. Sperline, S. Muralidharan, and H. Frieser **Appl. Spectrosc.** 40(7), 1019-22 (1986)

9. J. De Taeye and Th. Zeegers-Huyskens **Spectrosc. Lett.** 19(4), 299-310 (1986)

10. M. F. Vergnes, C. Grosset, and J. Alary **J. Chromatogr.** 455, 369-75 (1988)

11. D. J. Wells, B. M. Hanks, C. S. Yarbrough, and M. A. Davis, **J. Anal. Toxicol.** 12(3), 30-2 (1988)

12. T. B. Vree, L. Riemens, and P. M. Koopman-Kimenai **J. Chromatogr.** 428, 311-19 (1988)

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