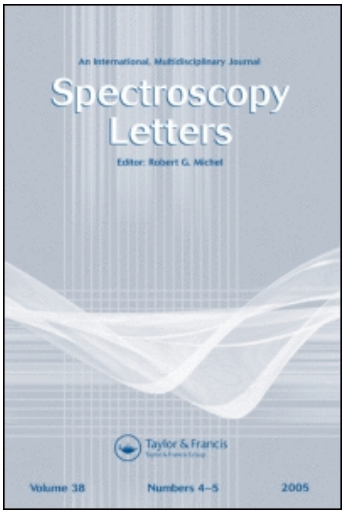

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

The Concept of Non-Dispersive Infrared Detector for Micro High Performance Liquid Chromatography Using KBr Buffer Memory Technique

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To cite this Article Jinno, Kiyokatsu(1981) 'The Concept of Non-Dispersive Infrared Detector for Micro High Performance Liquid Chromatography Using KBr Buffer Memory Technique', Spectroscopy Letters, 14: 10, 659 — 663
To link to this Article: DOI: 10.1080/00387018108062625
URL: http://dx.doi.org/10.1080/00387018108062625

SPECTROSCOPY LETTERS, 14(10), 659-663 (1981)

THE CONCEPT OF NON-DISPERSIVE INFRARED DETECTOR FOR MICRO HIGH PERFORMANCE LIQUID CHROMATOGRAPHY USING KBr BUFFER MEMORY TECHNIQUE

Key Words: Infrared Detector
Micro High Performance Liquid Chromatography
Non-Dispersive Concept
KBr Buffer Memory Technique

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Liquid Chromatography (LC) has gained acceptance as an analytical tool, and permits separations of mixtures of non-volatile compounds and species that are temperature sensitive.

Infrared spectroscopy(IR), on the other hand, has long been recognized as a qualitative tool for the chemist with its fingerprint region for molecular species. IR can be used either for detection in LC. In this way, this is limited to mobile phases that are transparent at the absorption wavenumber utilized. Normaly IR detector in LC [1), 2)] is set at one of the

wavenumber range that is characteristic absorption of functional groups. In this method, it is impossible to set at any wavenumber range if the characteristic absorption wavenumber of sample is unknown.

However there is another way to use IR detector in LC. This concept is the similar as the Total Ion Monitor technique in LC/MS combination. Total absorbance of samples separated from LC can be measured with a simple system of an IR source and a detector (for example, TGS, MCT) without gratings and filters to select any wavenumber (non-dispersive).

If it is possible to make this simple IR system, it seems to be very useful and convenient in LC detection for samples that have no absorption in UV region.

In this point, this communication is described the preliminary experiment of this consideration using Fourier Transform Infrared Spectrometer with a KBr buffer memory technique described elsewhere.[3)-5)]

A micro high performance liquid chromatograph used here was a JASCO FAMILIC 100N and UVIDEC 100 II system. Separation was performed on a JASCO SS-05 (5 μm porous silica) in a 0.5 mm i.d. x 15 cm teflon tube column with n-hexane/dichloromethane (65:35) as a carrier solvent.

IR measurements of absorption spectra were performed on a JEOL JTS-40X FT-IR Spectrometer equipped

with a 3x beam condenser, a 1 mm dia. aperture and a MCT detector. The test substances are 2,4-dinitrophenylhydrazones. A mixture of three 2,4-dinitrophenyl hydrazones (3 μg of each components) was introduced into a MHPLC column. The flow rate of carrier solvent is 5 μl/min. After chromatographic separation, the eluent passed through an UV flow cell and was continuously collected on a KBr crystal plate. (JASCO, 8.8 mm by 35 mm by 5 mm). At the end of collection, the buffer memory was automatically brought into the optical path of the FT-IR spectrometer, and IR absorption spectra were measured. These procedure was reported in previous works.[4), 5)] The absorbance of each sample components were calculated and stored in the computer files.

The chromatograms monitored on UV and FT-IR at the fixed wavenumbers and the wide range wavenumbers are shown in FIG. 1 (A), (B) and (C) respectively.

It is apparent that a little different shape each other is observed in the case of fixed wavenumbers' IR chromatograms because of the different absorption coefficients at each wavenumber. On the other hand, the chromatogram of IR at wide wavenumber range seems to be similar as that of UV.

This result gives us the important information that IR for MHPLC detector can be used by measuring the

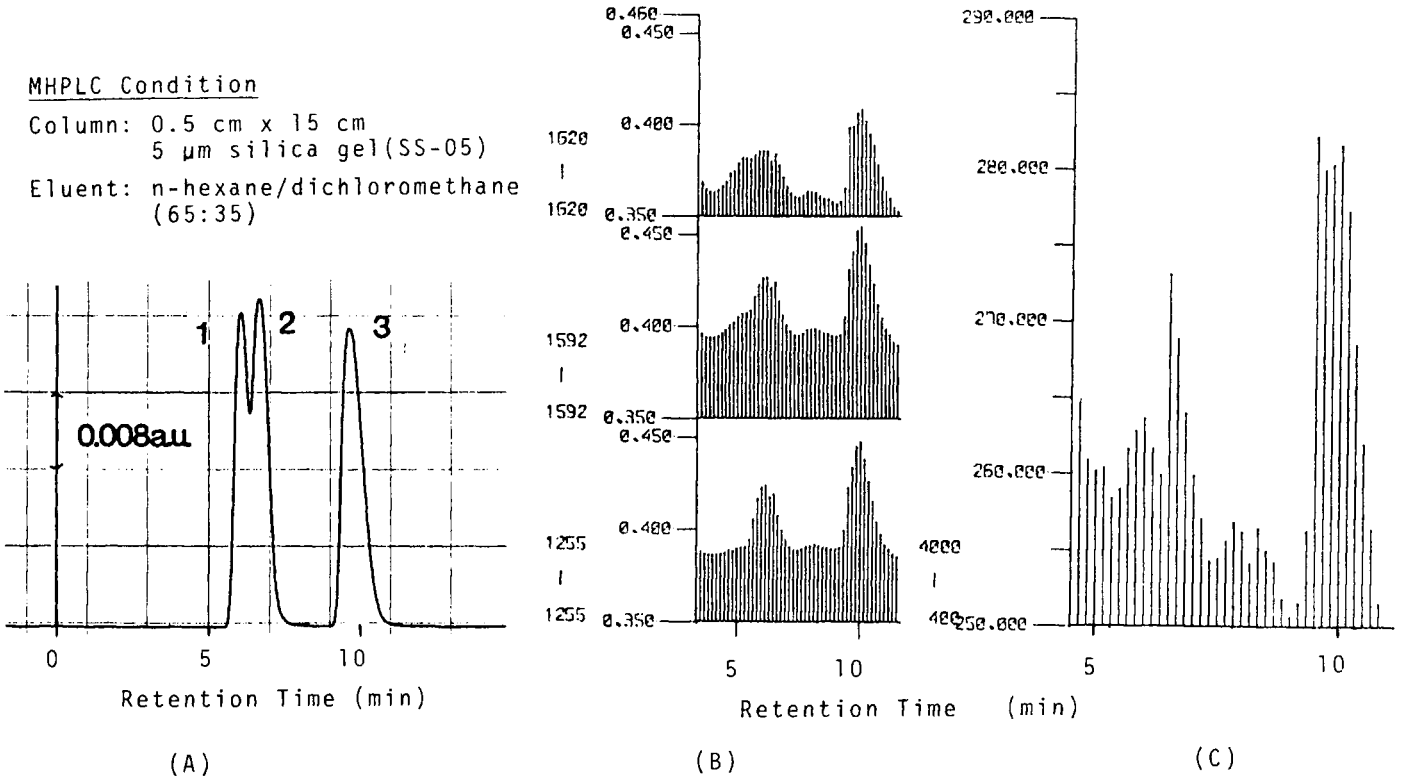

FIG. 1. Chromatograms monitored by UV and FT-IR
(A) UV Chromatogram at 254 nm.
(B) IR Chromatogram at 1620 cm^{-1}, 1592 cm^{-1} and 1255 cm^{-1}
(C) IR Chromatogram at 4000 cm^{-1}-400 cm^{-1} range.

Sample Components
1. di-n-amylketone-2,4-DNPH
2. di-n-propylketone-2,4-DNPH
3. acetone-2,4-DNPH

total absorbance in wide wavenumber range even if the characteristic absorption wavenumbers of sample are unknown. In the fact, this is not the best way to get the high sensitive detection with low noise signal but IR can be useful for the universal detector in LC based on this non-dispersive concept using KBr buffer memory technique.

ACKNOWLEDGEMENTS

The author would like to thank to C.Fujimoto for his assistance in this experiment.

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Received: July 16, 1981
Accepted: August 8, 1981