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Analysis of C₆₀ and C₇₀ fullerenes using high-performance liquid chromatography–Fourier transform infrared spectroscopy

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Abstract

The performance of Fourier transform infrared spectroscopy (FT-IR) detection coupled to high-performance liquid chromatography for the analysis of C₆₀ and C₇₀ fullerenes was investigated. The isocratic separation method involved an octadecylsilane (ODS) column and an acetonitrile–toluene (1:1) mobile phase. The hyphenated system was designed with a split valve to control eluent volume leading to the FT-IR detector; this allowed for additional coupling of the liquid chromatograph to ultraviolet–visible detection. On-line FT-IR spectra of C₆₀ and C₇₀ were matched with standard off-line FT-IR spectra from the literature. In addition, with band chromatograms individual fullerenes can be identified using FT-IR active modes known specifically for each fullerene. Few changes to a pre-existing HPLC–UV method were necessary for the HPLC–FT-IR method, and there was no need for fraction collection to identify the fullerenes C₆₀ and C₇₀. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

Since the discovery of fullerenes in carbon soot [1], many techniques have been utilized to identify the cage-like carbon structures. These identification techniques include infrared spectroscopy (IR) [2–9], nuclear magnetic resonance (NMR) [10–14], mass spectrometry (MS) [15–19], ultraviolet–visible spectroscopy (UV–Vis) [20,21], or a combination of these techniques [22–25]. Prior to identification, a separation of the desired fullerenes from the carbon soot mixture is often necessary.

At present, the separation technique most widely used is high-performance liquid chromatography (HPLC). Many HPLC methods have been developed [18–31]. Octadecylsilica (ODS) stationary phases are used most often [18–21,24–28], yet other

stationary phases are also available for fullerene separation [20,22–25,28–31].

The choice of mobile phase and stationary phase are important in fullerene separations by HPLC. With ODS stationary phases, a lack of selectivity is demonstrated by mobile phases made of pure solvents such as toluene, chlorobenzene, and 1-methylnaphthalene that have relatively high solubility for the fullerenes. However, often these stronger solvents can be mixed with a weaker solvent such as tetrahydrofuran, methanol, acetonitrile, or dichloromethane of lesser solubility power. With the selected solvent mixture for mobile phase and the appropriate ODS stationary phase, a reliable separation of fullerenes can be achieved. Good resolution can be accomplished while maintaining relatively good solubility [28].

Because off-line characterization of solutes for spectroscopy is labor intensive, the separation and

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identification of solutes “on-line” is preferred and a hyphenated chromatographic technique is used. On-line detection techniques minimize analysis time and prevent loss of sample, which is important for quantitative analysis. The most widely used techniques for on-line identification of fullerenes are HPLC–UV–Vis diode array detection [20–23] and HPLC–MS [18,19,22,23]. At present, FT-IR and NMR remain off-line identification techniques requiring collection and isolation of the fullerene fractions, usually on the preparative chromatographic scale [2–14,24,25]. Despite the existence of on-line HPLC–NMR and HPLC–FT-IR, the use of these techniques for fullerenes has not yet been reported in the literature.

On-line HPLC–FT-IR has recently become available for analytical separations. It is an important analytical tool that can enhance qualitative information about unknown solutes. This technique can be used in addition to other on-line chromatographic detection techniques such as UV–Vis, MS, and NMR. Since HPLC–FT-IR eliminates the need for fraction collection followed by characterization, quantitative analysis can be performed as well. In addition, the sample is not destroyed and can be further characterized using other techniques.

In this study, results from the analysis of fullerenes (C_{60} and C_{70}) using HPLC–FT-IR are obtained. Chromatographic separation by both HPLC–FT-IR and HPLC–UV–Vis systems are shown. FT-IR spectra and FT-IR band absorbance chromatograms of C_{60} and C_{70} showed that HPLC–FT-IR would be useful for the characterization of other fullerenes of other sizes, as well as fullerene derivatives.

2. Experimental

2.1. Materials

A fullerene mixture (approx. 70% C_{60} , 20% C_{70}) and C_{60} (99.5%) were obtained from the Southern Chemical Group (Tucker, GA, USA). C_{70} (98%) was donated by Yuri Gorfinkl of the Southern Chemical Group. HPLC-grade toluene and acetonitrile were obtained from Sigma (St. Louis, MO, USA).

2.2. Sample preparation

The fullerene samples obtained from the Southern Chemical Group were used as received, with no purification procedure. Samples were weighed out on a Mettler MT5 (Toledo, OH, USA) analytical balance. HPLC-grade (100%) toluene was used as the diluent for all samples.

2.3. HPLC with on-line UV detection

The liquid chromatography–visible detection system consisted of a pump, a reversed-phase column, a flow splitter, a UV–Vis detector, and an integrator (Fig. 1a). The pump was an LDC quaternary solvent delivery system equipped with a membrane degasser from Thermo Separation Products (San Jose, CA, USA). The 150 mm×4.6 mm I.D. separation column was packed with Ultracarb 5 μ m ODS stationary phase from Phenomenex (Torrance, CA, USA). The mobile phase was composed of an isocratic mixture of toluene–acetonitrile (1:1). Toluene was used as the marker of the void volume. The mobile phase flow-rate was set at 1.0 ml/min. Sample injections were performed manually with volumes ranging from 0.5 to 40 μ l. A split valve junction or flow splitter was used so that approximately 90% of the eluent flowed from the column to the UV–Vis detector. The eluent was monitored at a 325 nm wavelength using an LC-95 wavelength programmable UV–Vis spectrophotometer from Perkin-Elmer (Norwalk, CT, USA). Integration was performed using a Spectra-Physics SP4270 integrator from Thermo Separation Products.

2.4. HPLC with on-line Fourier transform infrared detection

FT-IR detection was performed on-line using the Fourier transform infrared chromatograph (IRC) from Bourne Scientific (Acton, MA, USA) (Fig. 1b) [32]. The IRC had a scan rate of 10 spectra/s. Approximately 30 spectra were co-added to obtain a resolution of 8 cm^{-1} . Data acquisition and analysis was performed with Grams/32 (Galactic Industries, NH, USA) modified software. The full spectral scan range was from 4000 to 650 cm^{-1} . Since the IRC was limited to low volume throughput, the splitter

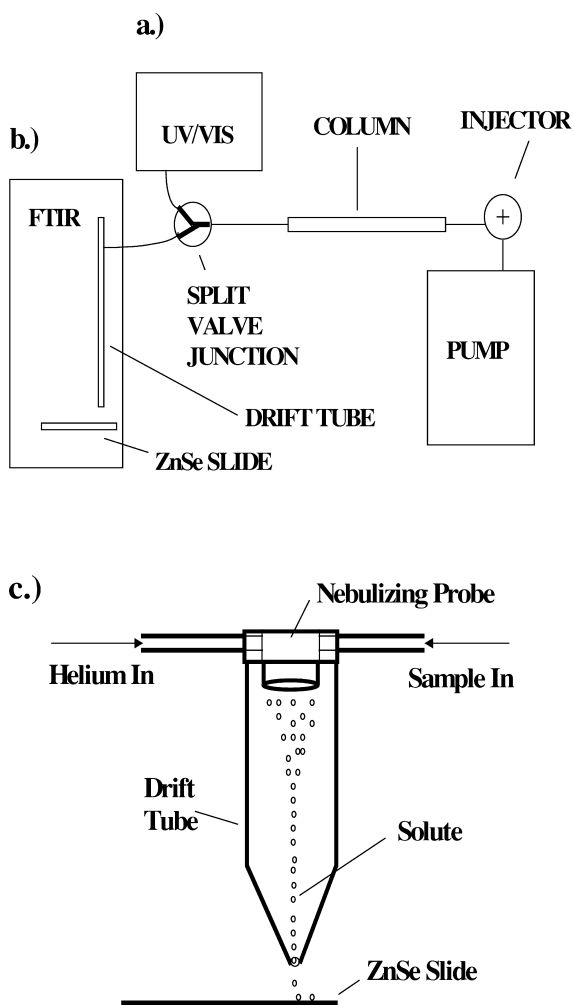


Fig. 1. Block diagram of the simultaneous (a) HPLC–UV and (b) HPLC–FT-IR on-line chromatographic system, and (c) schematic illustration of the drift tube interface, for solute deposition, in the Bourne IRC.

allowed approximately 10–20% of eluent to flow into the IRC. At the interface, the sample stream was nebulized, transported through a drift tube via helium, and deposited onto a zinc selenide (ZnSe) slide (Fig. 1c). The drift tube was set at 140°C to allow for vaporization of the mobile phase constituents (toluene, b.p. 120°C and acetonitrile, b.p. 76°C). The mobile phase conditions for HPLC–FT-IR were the same as those for HPLC–UV analysis.

3. Results

3.1. HPLC–UV analysis

Based on the method of Jinno et al. [21], the eluent was monitored at 325 nm. This wavelength was chosen because the absorbance associated with toluene at 254 nm interferes with the absorbance of both C_{60} and C_{70} . The HPLC–UV chromatogram of the fullerene separation is shown in Fig. 2. In less than 30 min, the C_{60} and C_{70} fullerenes were separated and had retention times of 15.7 and 27.9 min, respectively.

3.2. HPLC–FT-IR analysis

The fullerenes of C_{60} and C_{70} were deposited onto a zinc selenide slide and detected by FT-IR. Because a flow splitter was used, the C_{60} and C_{70} could be detected simultaneously by FT-IR and UV. The FT-IR intensity chromatogram was obtained and is shown in Fig. 3. The intensity chromatogram is defined as the recording of the highest absorbance output within the 4000 to 650 cm^{-1} range per unit time within a chromatographic separation. The average retention times for C_{60} and C_{70} were 16.2 and 28.7 min, respectively. The difference in retention times for C_{60} and C_{70} using on-line FT-IR and UV were due to the extra column tubing leading to the FT-IR detector.

In a comparative study of the HPLC–UV and HPLC–FT-IR techniques, there was a proportional difference in the response ratio of C_{70} and C_{60} . The HPLC–UV chromatogram of C_{60} and C_{70} showed a signal response ratio of $0.28 C_{70}/(C_{60}+C_{70})$ ($\sim 20\% C_{70}$, w/w). However, in the HPLC–FT-IR intensity chromatogram C_{70} has a higher absorbance signal than C_{60} . This corresponds to a signal response ratio of $0.67 [C_{70}/(C_{60}+C_{70})]$. The difference is a result of the higher absorbance intensity or molar absorptivity of C_{70} . Therefore, one should be aware and avoid misinterpretation of concentration from the direct response of fullerenes within an FT-IR intensity chromatogram.

3.2.1. C_{60} spectra

The chromatographic bands can be analyzed by their corresponding FT-IR spectra. For example, the

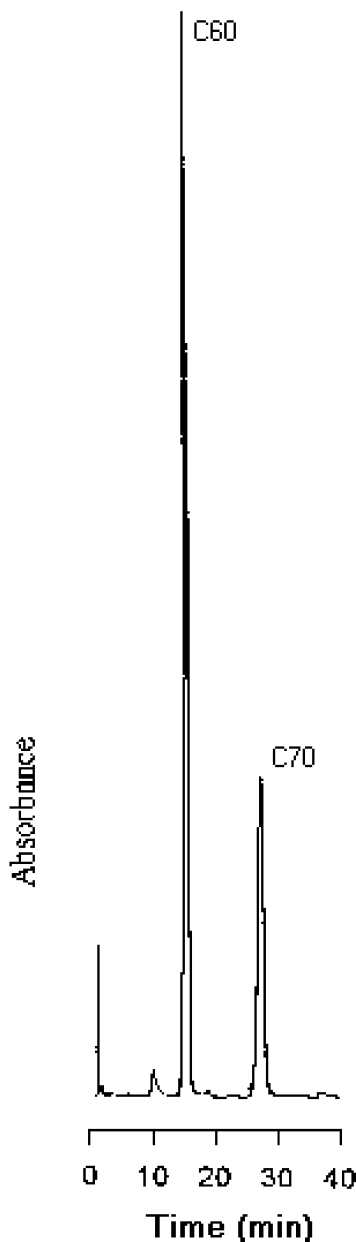


Fig. 2. Chromatogram of C_{60} and C_{70} using HPLC with ultra-violet (UV) detection at a wavelength (λ) of 325 nm. Mobile phase was toluene–acetonitrile (1:1) with a flow-rate of 1 ml/min. Manual injection of 40 μ l sample of fullerene mixture (1 mg/ml) of C_{60} and C_{70} .

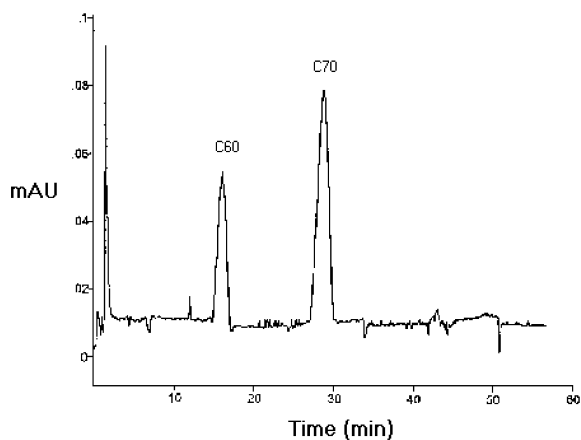


Fig. 3. HPLC–FT-IR intensity chromatogram of C_{60} and C_{70} . Mobile phase was toluene–acetonitrile (1:1) with a set flow-rate of 1 ml/min. Manual injection of 40 μ l sample of fullerene mixture (1 mg/ml) of C_{60} and C_{70} .

chromatographic band of the C_{60} fullerene separated from the fullerite mixture and retained at ~ 16 min (Fig. 3) had a corresponding FT-IR spectrum shown in Fig. 4a. The two major signals observed at 1429 and 1181 cm^{-1} are attributed to infrared allowed, dipole active vibrational modes of F_{1U} symmetry for C_{60} [25,33]. There are a total of four active vibrational modes, including two other FT-IR absorption bands at 576 and 527 cm^{-1} not shown due to detection limitation. These active vibrational modes are attributed to the icosahedral symmetry of the C_{60} molecule, which belongs to the icosahedral point group, defined as I_h [25,33]. The third major FT-IR band at 725 cm^{-1} is unassigned and has yet to be explained as to its association with C_{60} . However, this band has been observed by others (see Fig. 5) [25]. Fig. 5 shows the spectral matching of the three major FT-IR bands of C_{60} analyzed on-line (Fig. 5a) using the ZnSe slide at 8 cm^{-1} resolution and an offline spectrum of C_{60} (Fig. 5b) using a KBr substrate at 0.5 cm^{-1} resolution [25]. Examination of the spectra in closer detail reveal less intense FT-IR vibrational modes resulting from possible second-order combinations, such as the absorption bands observed at 2349, 2329 and 692 cm^{-1} (Fig. 4a). A spectral comparison of the HPLC–FT-IR spectrum of C_{60} with an off-line FT-IR spectrum performed by

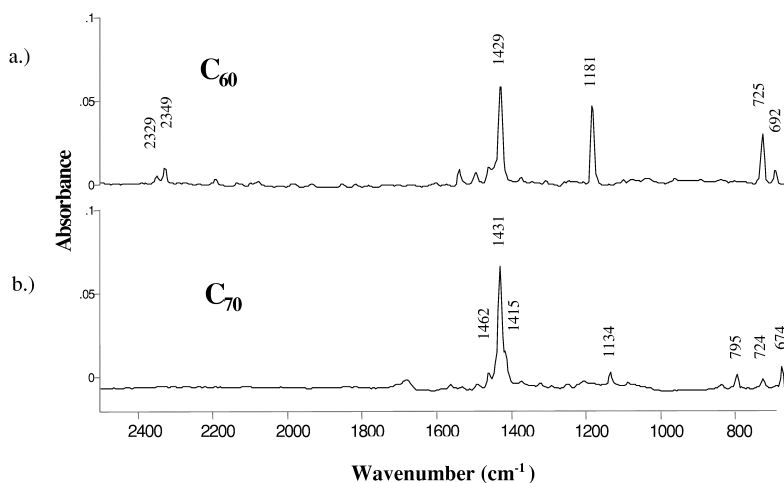


Fig. 4. HPLC-FT-IR absorbance spectra of C_{60} and C_{70} deposited onto a ZnSe slide. (a) C_{60} with major IR signals of F_{1U} symmetry at 1429 cm^{-1} and 1181 cm^{-1} and minor bands at 2329 , 2349 , 725 , 692 cm^{-1} . (b) C_{70} with a major IR signal at 1431 cm^{-1} and less intense bands at 1462 , 1415 , 1134 , 795 , 724 , and 674 cm^{-1} . Conditions: resolution was 8 cm^{-1} within the range of 2500 to 650 cm^{-1} .

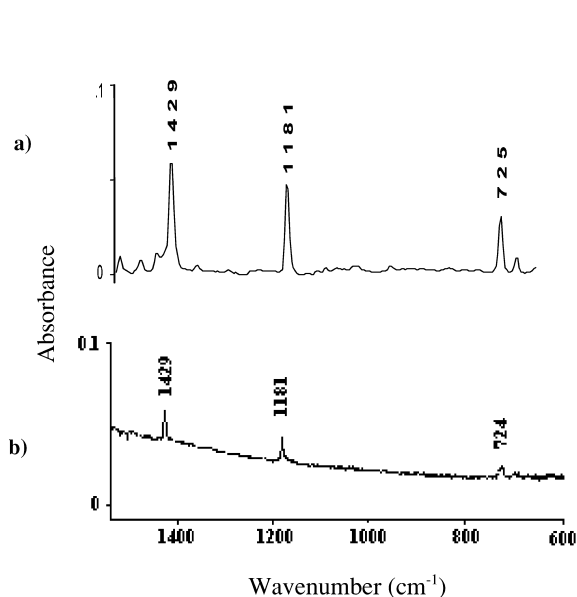


Fig. 5. C_{60} FT-IR spectral comparison between the absorbance spectrum of C_{60} deposited onto (a) ZnSe slide using on-line FT-IR with the resolution of 8 cm^{-1} and (b) KBr substrate using off-line FT-IR analysis with the resolution of 0.5 cm^{-1} , reprinted in part with the permission from Ref. [25]. Copyright 1991 American Chemical Society. Detection range was 1500 to 600 cm^{-1} . Major IR signals observed at 1429 , 1181 , and 725 cm^{-1} .

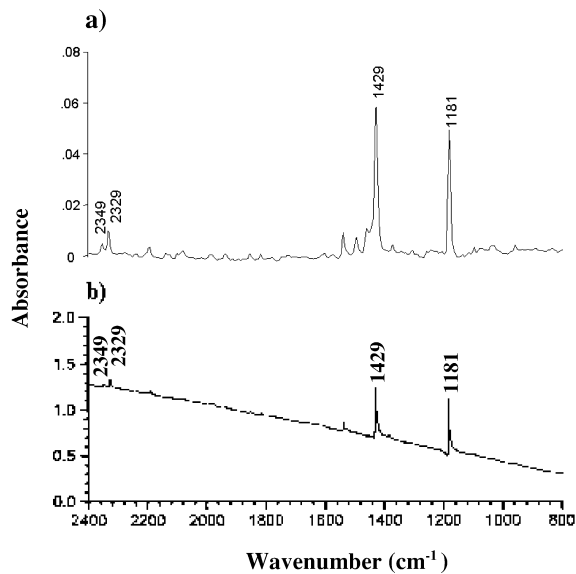


Fig. 6. Illustration of the second-order combination modes associated with C_{60} . FT-IR spectral comparison between the absorbance spectrum of C_{60} deposited onto (a) ZnSe slide using on-line FT-IR with the resolution of 8 cm^{-1} and (b) KBr substrate using off-line FT-IR analysis with the resolution of 0.5 cm^{-1} (reproduced with permission from Ref. [34]). Detection range of ~ 2400 to 800 cm^{-1} . Example of assigned second-order combination bands observed at 2349 and 2329 cm^{-1} .

Gadd et al. [34] demonstrate the similar combination bands, 2349 and 2329 cm^{-1} , observed (Fig. 6). In C_{60} IR spectra, some FT-IR vibrational modes are attributed to crystal field and isotope effects, which may explain the band observed at 725 cm^{-1} [35,36].

3.2.2. C_{70} spectra

More FT-IR spectral information could be obtained from the FT-IR intensity chromatogram shown in Fig. 3, by analyzing the FT-IR spectrum associated with the chromatographic band at ~ 29 min. The C_{70} fullerene separated from the fullerite mixture was verified by the on-line FT-IR spectrum and compared to off-line analysis. The on-line HPLC–FT-IR spectrum of C_{70} is shown in Fig. 4b. In the spectral region of 1600 to 650 cm^{-1} there are six active vibrational modes assigned to C_{70} . Those FT-IR bands are observed at 1431, 1462, 1415, 1134, 795, and 674 cm^{-1} , with an unassigned band at 724 cm^{-1} (Fig. 7a); similar FT-IR absorbance bands were observed by Jishi et al. (Fig. 7b) [36]. In their work, C_{70} was analyzed on a cesium iodide (CsI) substrate. The active vibrational modes observed in Fig. 7 are due to the symmetry of C_{70} , defined as a D_{5h} symmetry group. Because of the lower symmetry, there are more IR active vibrational modes as compared to C_{60} .

3.2.3. C_{60} and C_{70} band chromatograms

In addition to intensity chromatograms, individual fullerenes can be identified by the display of FT-IR band chromatograms. Band chromatograms are based on the fullerene's corresponding vibrational modes and can provide more information about each of the fullerenes than retention alone. Whereas the FT-IR intensity chromatogram displays the most intense signal at each time point, a band chromatogram is displayed by the intensity of an assigned wavenumber throughout the time range. In order to acquire useful band chromatograms, specific vibrational modes unique to each solute, should be known. For example, the active vibrational modes observed by C_{60} and C_{70} . Specific vibrational modes are used to assure absorbance differences of solutes in the chromatogram. The differences in the FT-IR spectra of C_{60} and C_{70} are shown in Fig. 4.

The C_{60} fullerene was identified by observing the FT-IR spectra band chromatograms at 1181, 725, and

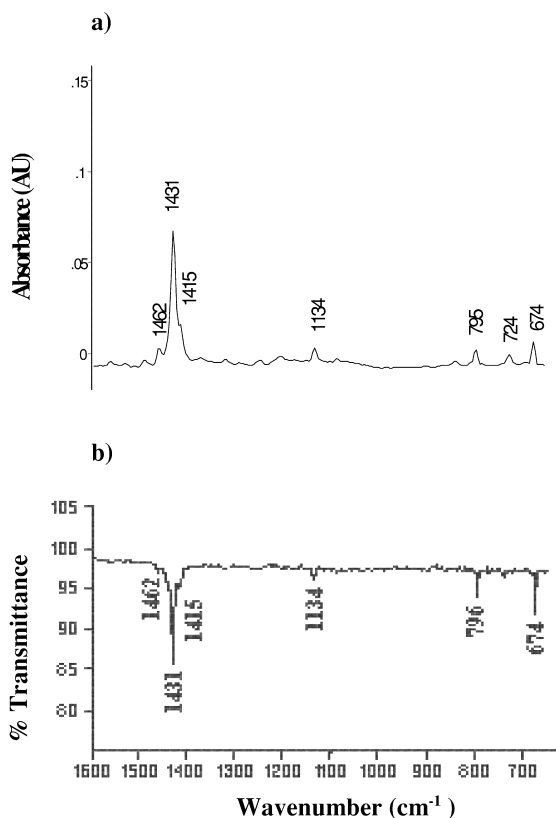


Fig. 7. Illustration of the major active vibrational modes associated with C_{70} . FT-IR spectral comparison between the absorbance spectrum of C_{70} deposited onto (a) ZnSe slide using on-line FT-IR with the resolution of 8 cm^{-1} and a (b) CsI substrate using off-line FT-IR analysis, resolution not given (reproduced with permission from Ref. [36]). Detection range of 1600 to 650 cm^{-1} . Six of 11, strong active vibrational modes for C_{70} are shown: major signal at 1431 cm^{-1} and less intense bands at 1462, 1415, 1134, 795, and 674 cm^{-1} .

692 cm^{-1} . These band chromatograms for C_{60} are illustrated in Fig. 8a. As shown, the band chromatograms illustrate strong absorption for C_{60} and weak or no absorption for C_{70} (retained at ~ 29 min). The C_{70} fullerene was identified by assigning the wavenumbers 1134, 795, and 674 cm^{-1} as band chromatograms. These absorption bands, unique to the C_{70} fullerene, are illustrated in Fig. 8b. As shown, the chromatograms illustrate strong absorption bands for C_{70} whereas weak or no absorption is observed for C_{60} .

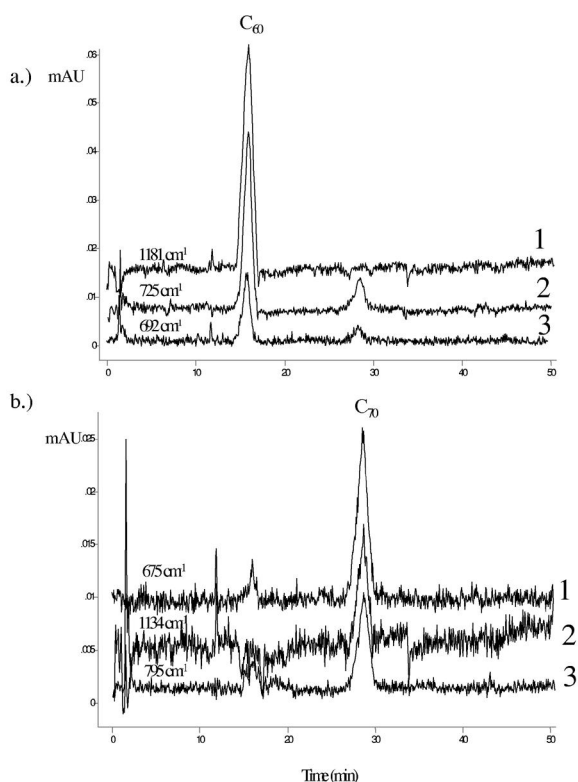


Fig. 8. (a) HPLC–FT-IR band chromatograms of C₆₀ fullerene at (1) 1181 cm⁻¹, (2) 725 cm⁻¹ and (3) 692 cm⁻¹. (b) HPLC–FT-IR band chromatograms of C₇₀ fullerene at (1) 674 cm⁻¹, (2) 1134 cm⁻¹ and (3) 795 cm⁻¹.

3.2.4. Separation performance

The separation performance of HPLC–FT-IR system was compared to that of the HPLC–UV system. Despite differences in retention times, retention factors were the same. A twofold decrease in the resolution and efficiency for the HPLC–FT-IR system, was a result of dispersion within the drift tube.

Linearity, reproducibility, and limit of detection were determined for the HPLC–FT-IR analysis of C₆₀ and C₇₀. Samples ranging in concentration from 29 to 131 ng and 1 to 2 μg, were used to determine linearity of C₆₀ and C₇₀ with correlation factors of 0.991 and 0.986, respectively. Twelve replicate injections of C₆₀ and C₇₀ at mass concentrations within the linearity ranges had less than 1.5% RSD. The limit of detections for C₆₀ and C₇₀ were determined to be 29 and 376 ng, respectively.

4. Conclusion

HPLC–FT-IR is a viable technique in the analysis of fullerenes. The fullerene species C₆₀ and C₇₀ were separated by reversed-phase HPLC and identified by FT-IR detection. No changes in the pre-existing separation method were necessary to adapt to the HPLC–FT-IR. Spectral matching and band chromatograms demonstrated the ability to recognize each fullerene's FT-IR characteristics and chromatographic retention. Identification of the fullerenes was possible without the need for the collection of fractions, which eliminated any possible loss of sample. Results showed that qualitative analysis of fullerenes in mixtures could be performed. The additional qualitative information obtained using the FT-IR makes the technique potentially useful for fullerene research applications. Future research includes investigations to determine if other fullerenes such as C₇₈, C₈₂, and C₈₄ can be separated and identified using HPLC–FT-IR.

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References

- [1] H.W. Kroto, J.R. Heath, S.C. O'Brien, R.F. Curl, R.E. Smalley, *Nature* 318 (1985) 162.
- [2] W. Krätschmer, L.D. Lamb, K. Fostiropoulos, D.R. Huffman, *Nature* 347 (1990) 354.
- [3] R. Meilunas, R.P.H. Chang, S. Liu, *J. Appl. Phys.* 70 (1991) 5128.
- [4] D.R. Lawson, D.L. Felheim, C.A. Foss, *J. Phys. Chem.* 96 (1992) 7175.
- [5] B. Chase, N. Herron, E. Holler, *J. Phys. Chem.* 96 (1992) 4262.
- [6] G. Orlandi, F. Zerbetto, P.W. Fowler, *J. Phys. Chem.* 97 (1993) 13575.
- [7] H. Yamawaki, M. Yoshida, Y. Kadodate, *J. Phys. Chem.* 97 (1993) 11161.
- [8] K. Kamaras, D.B. Tanner, L. Forro, *J. Supercond.* 8 (1995) 621.

- [9] A. Watanabe, O. Ito, M. Watanabe, *J. Phys. Chem.* 100 (1996) 10518.
- [10] C.S. Yannoni, P.P. Bernier, D.S. Bethune, *J. Am. Chem. Soc.* 113 (1991) 3190.
- [11] R.D. Johnson, C.S. Yannoni, J.R. Salem, G. Meijer, D.S. Bethune, in: *Fullerenes: Synthesis, Properties, and Chemistry*, American Chemistry Society, Washington, DC, 1992, p. 107, Chapter 7.
- [12] R. Baum, *Chem. Eng. News* 72 (1994) 40.
- [13] M. Saunders, H.A. Jimenez-Vazquez, B.W. Bangerter, *J. Am. Chem. Soc.* 116 (1994) 3621.
- [14] M. Saunders, H.A. Jimenez-Vazquez, R.J. Cross, *J. Am. Chem. Soc.* 117 (1995) 93050.
- [15] R. Malhotra, D.S. Ross, *J. Phys. Chem.* 95 (1991) 4599.
- [16] P. Wurz, K.R. Lykke, M.J. Pellin, *Vacuum* 43 (1992) 381.
- [17] W.R. Creasy, J.A. Zimmerman, R.S. Ruoff, *J. Phys. Chem.* 97 (1993) 973.
- [18] K. Jinno, T. Uemura, H. Nagashima, K. Itoh, *J. High Resolut. Chromatogr.* 15 (1992) 627.
- [19] J.F. Anacleto, R.K. Boyd, M.A. Quilliam, *J. High Resolut. Chromatogr.* 16 (1993) 85.
- [20] K. Jinno, H. Ohta, Y. Saito, T. Uemura, H. Nagashima, K. Itoh, Y. Chen, G. Luehr, J. Archer, J.C. Fetzer, W.R. Biggs, *J. Chromatogr.* 648 (1993) 71.
- [21] K. Jinno, T. Uemura, H. Ohta, H. Nagashima, K. Itoh, *Anal. Chem.* 65 (1993) 2650.
- [22] J.F. Anacleto, M.A. Quilliam, *Anal. Chem.* 65 (1993) 2236.
- [23] H. Richter, K. Taghizadeh, W.J. Grieco, A.L. Lafleur, J.B. Howard, *J. Phys. Chem.* 100 (1996) 19603.
- [24] H. Ajie, M.M. Alvarez, S.J. Anz, R.D. Beck, F. Diederich, K. Fostiropoulos, D.R. Huffman, W. Krätschmer, Y. Rubin, K.E. Schriver, D. Sensharma, R.L. Whetten, *J. Phys. Chem.* 94 (1990) 8630.
- [25] D.M. Cox, S. Behal, M. Disko, S.M. Gorun, M. Greaney, C.S. Hsu, E.B. Kollin, J. Millar, J. Robbins, W. Robbins, R.D. Sherwood, P. Tindall, *J. Am. Chem. Soc.* 113 (1991) 2940.
- [26] Y. Cui, S.T. Lee, S.V. Olesik, W. Flory, M. Mearini, *J. Chromatogr.* 625 (1992) 131.
- [27] J.J. Harwood, G. Mamantov, *J. Chromatogr. A* 654 (1993) 315.
- [28] Y. Wu, Y. Sun, Z. Gu, Q. Wang, X. Zhou, Y. Xiong, Z. Jin, *J. Chromatogr.* 648 (1993) 491.
- [29] C.E. Kibbey, M.R. Savina, B.K. Parseghian, *Anal. Chem.* 65 (1993) 3717.
- [30] J. Xiao, M.R. Savina, G.B. Martin, *J. Am. Chem. Soc.* 116 (1994) 9341.
- [31] K. Kimata, T. Hirose, K. Moriuchi, *Anal. Chem.* 67 (1995) 2556.
- [32] S. Bourne, *Am. Lab.* 30 (1998) 17F.
- [33] H. Kuzmany, R. Winkler, T. Pichler, *J. Phys. Condens. Matter.* 7 (1995) 6601.
- [34] G.E. Gadd, M. James, S. Moricca, D. Cassidy, P.J. Evans, B. Collins, R.S. Armstrong, *J. Phys. Chem. Solids* 59 (1998) 1383.
- [35] K.-A. Wang, A.M. Rao, P.C. Eklund, M.S. Dresselhaus, M.S. Dresselhaus, *Phys. Rev. B* 48 (1993) 11375.
- [36] R.A. Jishi, M.S. Dresselhaus, G. Dresselhaus, K. Wang, P. Zhou, A.M. Rao, P.C. Eklund, *Chem. Phys. Lett.* 206 (1993) 187.