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A Comparison between Different Acquisition Modes for FT-IR Spectra Collection from Human Cell Lipid Extracts †

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Abstract: Lipids are organic compounds that contribute to numerous cellular functions. Fourier Transform Infrared spectroscopy can be particularly useful in investigating the biochemical features of the lipid content of cells and their changes induced by interaction with physicochemical external agents. In the present work, we aim to investigate the extract of lipids from human cells to compare the results obtained by using two different geometries: transmission and attenuated total reflectance. Multiple acquisitions of spectra were carried out and statistical criteria were applied for monitoring and comparing them. The positive and negative aspects of the two examined acquisition modes are presented and discussed.

Keywords: FT-IR spectroscopy; transmission; ATR; lipids; hepatocarcinoma cells

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1. Introduction

Lipids are organic compounds widely distributed in nature and represent one of the four main classes of organic compounds of biological interest, along with carbohydrates, proteins, and nucleic acids. In eukaryotes, lipids contribute to numerous cellular functions, ranging from energy storage to cell signaling [1]. Thanks to its ability to analyze cellular components at a molecular level, Fourier Transform Infrared Spectroscopy (FT-IR) can be particularly useful in investigating the biochemical features of the lipid content of cells and their changes induced by interaction with physicochemical external agents. In the present work, we aim to investigate lipids extracted from cells to compare the results obtained by using two different geometries that are usually available for the acquisition of FT-IR spectra for liquid samples: transmission and Attenuated Total Reflectance (ATR) geometry [2]. Multiple acquisitions of spectra were carried out, and statistical criteria were applied for monitoring and comparing them. The positive and negative aspects of the two examined acquisition modes are presented and discussed.

2. Materials and Methods

Human hepatocarcinoma cells (HepG2) were cultured in Dulbecco's Modified Eagle Medium. The medium was supplemented with 10% heat-inactivated FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, and 1% L-glutamine. The cells were grown in a humidified atmosphere of 95% air/5% CO_2 at 37 °C in T25 flasks.

Lipids were extracted from cells by using the Bligh and Dyer method [3]. In this method, for each 1 mL of sample, 3.75 mL 1:2 (*v*/*v*) CHCl3:MeOH were added and vortexed thoroughly. Then, 1.25 mL $CHCl_3$ was added and vortexed again. Finally, 1.25 mL of deionized water (dH2O) was added, vortexed, and the sample was then centrifuged at 1000 rpm for 5 min at room temperature to yield a two-phase system (aqueous top and organic bottom). The organic bottom layer was carefully collected using Pasteur pipettes into a clean glass vial, dried with nitrogen, and stored at −20 °C until measurements.

To obtain measurements in transmission geometry, a few microliters of lipids extracted from hepatocarcinoma cells (HepG2) were dissolved in methanol and positioned onto CaF_2 windows. Spectra were then acquired using the microscope stage of a Perkin Elmer Spectrum One spectrometer, which was equipped with a mercury cadmium telluride (MCT) detector. This approach allowed for the collection of spectra using 32 scans in the range from 4000 to 1000 cm^{-1} with a 4 cm^{-1} spectral resolution in a 100 × 100 μm^2 region. For measurements in Attenuated Total Reflectance (ATR) geometry, drops of the extracted lipids were placed on the top of the diamond crystal of the Universal ATR accessory of the abovementioned FT-IR spectrometer provided via an MIR TGS detector. In this case, spectra were collected using 32 scans in the range from 4000 to 650 cm^{-1} with a 4 cm^{-1} spectral resolution.

Preliminary subtraction of the background spectrum acquired in a free-cell zone of the slide was performed for all the spectra. For the purpose of comparing spectra, Standard Normal Variate (SNV) normalization was carried out [4]. To compare the acquisition modes, a study of the correlation among the various spectra acquired in transmittance and ATR mode was carried out using a MATLAB code.

3. Results and Discussion

In Figures 1 and 2, the average FT-IR spectra for lipids extracted from HepG2 cells are reported. For the spectrum collected in transmission mode, the investigated range is from 4000 to 1000 cm^{-1}, while for the spectrum acquired in ATR mode, the spectrum is related to the 4000–650 cm^{-1} wavenumber region. As can be observed, the two spectra exhibit very similar behavior in the high-wavenumber region, while some differences occur in the fingerprint region.

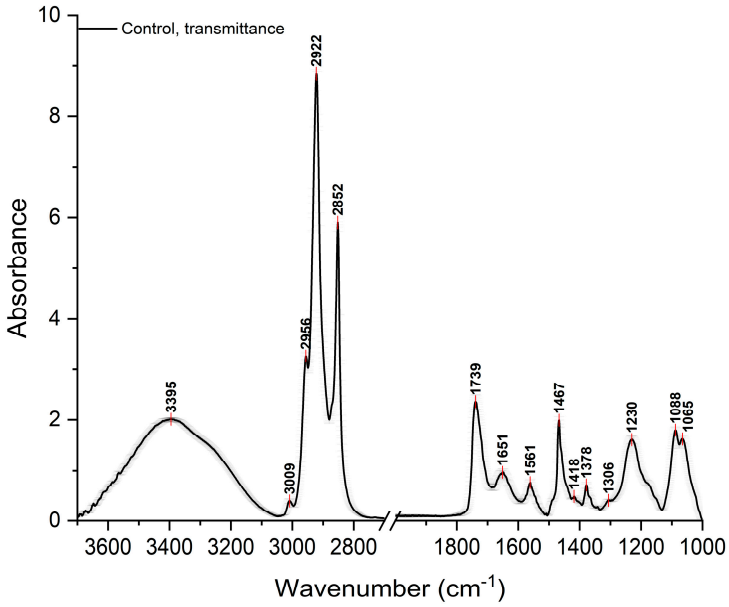

Figure 1. Average FT-IR spectrum obtained in transmittance mode from the average spectra relative to repeated measurements on lipids extracted from HepG2 cells. Data are presented as mean ± SEM. The peaks are shown, and the assignments are made by referring to texts in the literature (Table 1) [5–7].

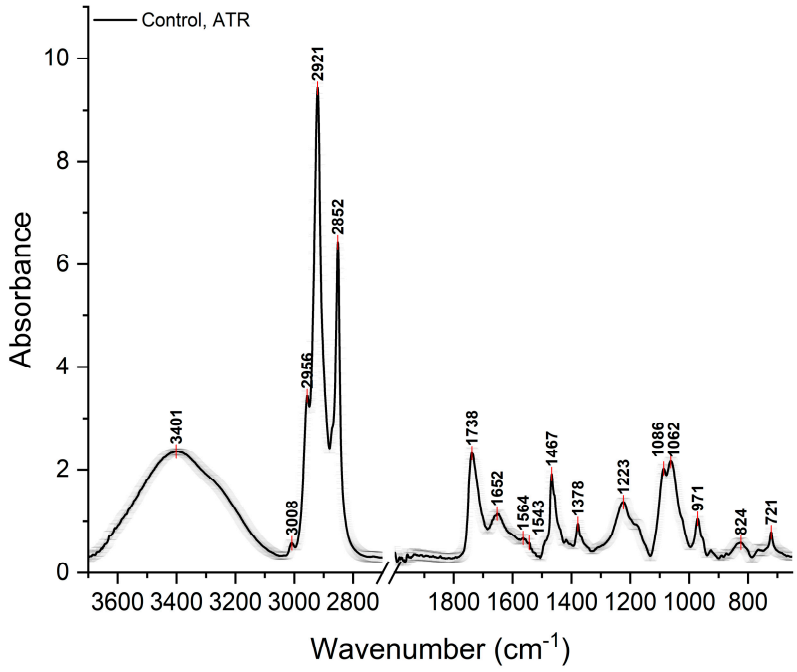

Figure 2. Average FT-IR spectrum obtained in ATR mode from the average spectra relative to repeated measurements of lipids extracted from HepG2 cells. Data are presented as mean ± SEM. The peaks are shown, and the assignments are made by referring to texts in the literature (Table 1) [5–7].

Table 1. Spectral position of main peaks of FT-IR spectra of Figures 1 and 2. Assignments are reported according to refs. [5,8,9]. Bold character indicates shifts larger than experimental spectral resolution (4 cm^{-1}). Nomenclature: ν stands for stretching, s = symmetric, as = asymmetric, δ stands for bending.

Assignments	Transmittance	ATR
	Peak (cm^{-1})	**Peak (cm^{-1})**
ν(N-H), ν(O-H), ν(C-H)	3395	**3401 (+6)**
ν(H-C=)	3009	3008 (−1)
νas(CH_3)	2956	2956
νas(CH_2)	2922	2921 (−1)
νs(CH_2)	2852	2852
ν(C=O)	1739	1738 (−1)
ν(C=O)	1651	1652 (+1)
δ(N-H)	1561	**1543 (−18)**
δ(CH_2)	1467	1467
δ(CH_3)	1378	1378
–	1306	–
νas(PO_2^-)	1230	**1223 (−7)**
νs(PO_2^-)	1088	1086 (−2)
ν(C − O − H)	1065	1062 (−3)
ν(C − O − P)	–	–
$N^+(-CH_3)_3$	–	971
νas(P-O)	–	824
ρ(CH_2)	–	721

In Table 1, the positions of the main contributions are reported together with their assignments. Upon examining the peak positions of the bands of interest, the spectra acquired in the two modes appear consistent. The positions are almost similar, and in general comparable, since the differences are lower than the spectral resolution of the measuring apparatus, i.e., 4 cm^{-1}, except for the bands around 3401 and 3395 cm^{-1} (related to the asymmetric stretching of the O-H, N-H and C-H groups) that show a shift of +6 cm^{-1}, the features at 1561 and 1541 cm^{-1} (due to the N-H bending of amide II group) that show a shift of −18 cm^{-1} and the peaks at 1230 and 1223 cm^{-1} (ascribed to the asymmetric stretching of the phosphate group), which evidence a shift of −7 cm^{-1} in the spectra associated with the two acquisition methods.

The major differences between the two acquisition modes lie in the sample preparation and the extension of the infrared range characteristic of the spectrum. As described in the Material and Methods section, samples for transmittance measurements were first diluted in methanol and then left to dry on CaF_2 windows; in contrast, samples for ATR, also dissolved in methanol, were placed directly on the diamond core and, also in this case, were left to dry. In the ATR mode, it is possible to obtain spectra of up to 650 cm^{-1}, instead of up to 1000 cm^{-1} as in transmittance. In general, the high wavenumber region is less dependent on the acquisition geometry compared with the fingerprint region.

We also investigated the spectra reproducibility given by the two different acquisition modes. From the correlation study between the various spectra acquired repeatedly in transmittance, the analysis returned a correlation coefficient R = 0.95 in the case of the high wave number range (3700–2700 cm^{-1}), and R = 0.79 in the case of the fingerprint zone (1800–1000 cm^{-1}). In the case of attenuated total reflectance (ATR) acquisition, the correlation coefficient obtained in both high and low wave numbers is R = 0.99. This analysis indicated that ATR geometry can be the most valuable approach for acquiring infrared spectra from lipid samples according to the literature [10].

4. Conclusions

The present study indicates that both transmittance and ATR acquisition geometries can be adopted for the investigated lipid sample, but ATR approach offers some advantages in terms of wavenumber range and spectra reproducibility. This can probably be due to the different sampled region. In transmission mode, the spectra were acquired on small regions (100 × 100 μm^2), while in ATR geometry, the spectra were acquired by exploiting the total area of the diamond crystal surface (nearly equal to 3 mm^2). In addition, this measurement method speeds up the acquisition of spectra and reduces the costs.

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