Amyloid Detection Using a Peltier-Based Device

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Amyloid aggregation of polypeptides is related to a growing number of pathologic states known as *amyloid disorders*. At present, it is clear that any proteins submitted to appropriate physicochemical environment can acquire fibrilar conformation. Fourier transform infrared spectroscopy (FTIR) has been a widely used technique to study temperature-induced amyloid-fibrils formation in vitro. In this way, strict changes and temperature controls are required to characterize the physicochemical basis of the amyloid-fibrils formation. In this article, the development of a highly efficient and accurate Peltier-based system to improve FTIR measurements is presented (see "An Old Physics Phenomenon Applied to a Serious Biomedical Pathology." The accuracy of the thermostatic control was tested with biophysical parameters on biological samples probing its reproducibility. The design of the present device contributes to maintain the FTIR environment stable, which represents a real contribution to improve the spectral quality and thus, the reliability of the results.

Background

In the brain, proteins and peptides, such as α-synuclein [1], β-amyloid peptide [2], transthyretin [3], and others, can aggregate to form long and thin needle-shaped structures called *fibrils*. Their accumulation gives rise to incurable and fatal dementia disorders, such as Alzheimer's, Parkinson's, and Creutzfeldt–Jakob diseases. Currently, an increasing number of amyloidogenic precursors have been identified [4], [5], although the detailed mechanism underlying the formation of fibrils by these soluble proteins still remains unclear. The inhibition (or perhaps reversion) of amyloid-fibril formation has been suggested as a possible preventive mechanism to amyloid diseases. Thus, many efforts are under way to reach a full understanding of the molecular processes involved. The examination of the in vitro formed fibrils with circular dichroism, FTIR, and electron microscopy has showed that they are completely similar to those found in amyloid plaques in vivo. In this way, in vitro amyloid formation process has been extensively studied, and now it is clear that each protein requires an appropriate physicochemical environment to be properly folded or to acquire the fibrilar form [6], [7].

FTIR has been a widely used technique to study temperature-induced amyloid-fibrils formation in vitro.

FTIR is a technique currently used to study the in vitro amyloid-fibrils formation since specific infrared bands have been assigned to their characteristic interchain β-aggregation. Thermal unfolding of proteins with the subsequent β-aggregation can be induced in some proteins with a controlled thermal stress. Therefore, this is one of the most efficiently handled methods to study the molecular basis of the amyloid-fibrils formation. However, high-quality results require FTIR spectra with excellent signal-to-noise ratio together with adequate and accurate thermal change records. The signal-to-noise ratio depends directly on the spectrophotometer resolution and on the biological sample concentration. In addition, it is inversely related to the water vapor present in the sample compartment.

This article aims to develop a device capable of controlling the temperature changes within the FTIR sample compartment by using the temperature slope versus time (TST) system. The size of the device must be small enough to reduce interferences with the spectrophotometer measure and/or purge systems in an FTIR experiment. The device must also automatically test and assign every temperature to a corresponding FTIR spectrum. The accuracy of the thermostatic control and the TST system was tested with biological samples. Moreover, the biophysical parameters along with the device reliability and accuracy were also determined.

System Description

In 1834, Jean Charles Peltier discovered that an electric current, when crossing the junction of two different metals, produces heat on one side while removing heat from the other side (thus, it generates a temperature gradient), with heat being proportional to the current (see "An Old Physics Phenomenon Applied to a Serious Biomedical Pathology" by Max E. Valentinuzzi). The proportionality constant (or Peltier coefficient) depends on materials and represents how much heat is carried per unit charge. One way to understand how this effect could cool a junction is to note that when electrons flow from a region of high density to a region of low density, they expand (as with an

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An Old Physics Phenomenon Applied to a Serious Biomedical Pathology

Max E. Valentinuzzi

Biochemistry became an essential discipline in medical practice, and as it evolved along with other scientific branches, someone once dared to say that everything had been discovered, while bringing up the legendary phrase "nothing new under the sun" (*nihil novum sub sole*) to strengthen the assertion. Without backing up the latter, the truth is that it is becoming more difficult than before to come up with new dashing ideas. In addition, many of the recent inventions and their consequent applications often rely on principles created a long time ago. An example can be found in many numerical methods developed by brilliant mathematicians, such as Newton, Gauss, Leibniz, or Lagrange, to solve problems that were essentially unmanageable with continuous tools, underlining the even more basic breakthrough role of George Boole's 19th century algebra for the logical design of 20th century computers. Herein, the authors of this column take advantage of the old Peltier effect to develop an instrument for the spectroscopic analysis of amyloid fibrils in biological samples, remarking the current significance in the health-care system for the detection of devastating disorders, such as Alzheimer's, Parkinson's, and Creutzfeldt–Jakob diseases, and as a new contribution from biomedical engineering.

Biochemistry: When Did It Make Its Appearance?

In 1851, Henry Bence Jones (1813–1873), an English physician and chemist, looked back to Lavoisier's experiments as a symbol of the first link between physiology and chemistry. Physicians needed to understand the workings of the body in chemical terms, so that medical chemistry had to be regarded as one of the earliest roots of chemical physiology or biochemistry in the current terminology. However, and in spite of its current outstanding importance, chemistry was pushed to the periphery of medical science until the 19th century [S1]. German Physician Johann Ludwig Wilhelm Thudichum (1829–1901), who in 1853 moved to London (where he worked for the remainder of his career), was a pioneer in the field of neurochemistry and a founder of brain chemistry. He believed that no pathology was possible before a full analysis of all the body tissues in their healthy state had been achieved; his results and ideas were not well received by his contemporaries [S2]. The term *biochemistry* was apparently introduced in 1903 by Carl Alexander Neuberg (1877–1956), another German chemist; however, it is accepted that its usage started more than 400 years ago [S3]. This important and essential discipline in medical practice makes use of physicochemical principles and laws and technological knowledge; the Peltier effect is an excellent example.

What Is the Peltier Effect?

It was an inadvertent discovery made by the French physicist Jean Charles Athanase Peltier (1785–1845) while investigating properties of electricity [S4]–[S6]. In an experiment, Peltier joined copper wire and bismuth wire together and connected them to each other and then to a battery (how he came up with the idea belongs to the mysteries of intellectual creation). When current was allowed to flow, one of the junctions of the two wires got hot, while the other junction got cold, and thus it generated a temperature gradient, with heat being proportional to the current flow.

The proportionality constant (or Peltier coefficient) depends on materials and represents how much heat is carried per unit charge. One way to understand how this effect could cool a junction is to note that when electrons flow from a region of high density to low density, they expand (as with an ideal gas) and cool. A Peltier cooler/heater is a solid-state active heat pump that transfers heat from one side of the device to the other. If the cold junction was put inside an insulated box, it became a low-efficiency refrigerator. Often, this phenomenon is coupled with its inverse, the Seebeck effect, and the two can be related to a third, the Thomson effect. All three together constitute different components of the same scientific principle.

In essence, the Peltier effect represents a heat difference at the junction of two different metals when connected together. It is the basis of the small 12/24 V heaters/coolers sold for use in vehicles. By switching the direction of the current, either heating or cooling may be obtained. It also forms the basis of the stable junction-heated soldering irons and also finds use for spot cooling of some integrated circuits. Heat flows from one junction to the other. One side gets hot and the other side cold. In addition, a heat dissipation device must be attached to the hot side to keep a cooling effect on the cold side. Interestingly enough, Peltier never went through any formal academic education; he worked first as a clockmaker for the firm A.L. Bréguet. Upon receiving an inheritance in 1815, he became interested in science, producing contributions in thermoelectricity, electromagnetism, and meteorology. In 1834, he discovered the effect that would later be named after him. He also developed and improved a number of electrical measuring instruments, including the electrometer.

In this column, the authors examine a Peltier unit together with an electronic controller and heating exchange system that were used as thermostatic control along with the TST system suitable to be used in FTIR experiments (please note the bioengineering concepts employed herein).

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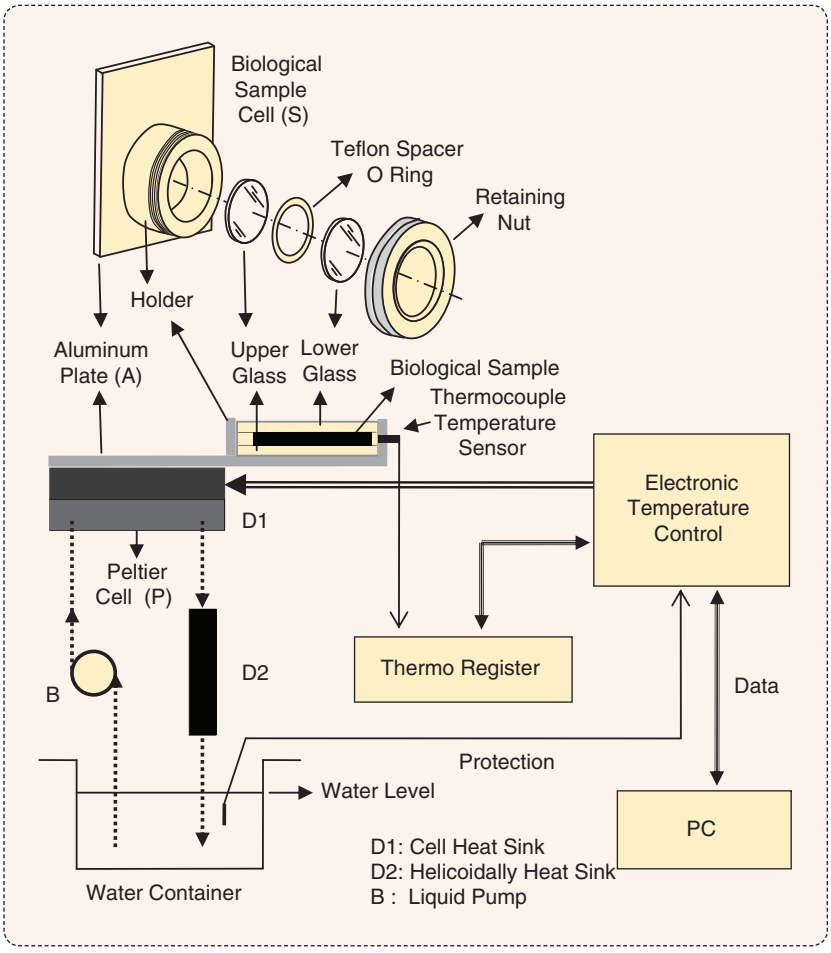

FIGURE 1 A schematic diagram of the sample cell and the temperature control system, constituted by a Peltier cell (P), temperature sensor, cooling system, protection, temperature register, electronic temperature control, and a PC with a dedicated software to read the data and operate over the temperature control.

ideal gas) and cool. A Peltier cooler/heater is a solid-state active heat pump that transfers heat from one side of the device to the other side.

A Peltier unit together with an electronic controller and a heating exchange system was used as thermostatic control along with the TST system suitable to be used in FTIR experiments. Through adequate continuous temperature control over the cell, it is possible to modify the temperature on one face of the cell while the other one is at quasi constant temperature. Temperature on the cell S is evaluated by a mini thermocouple (K-type), and this information is fed into a microcontrolled circuit while the current on the cell is modulated by it. To generate the programmed positive temperature slopes (2, 1, 0.5, 0.33, or 0.25 °C/min), a direct current pulse with modulation is used. The operating range is divided into three steps (10–15, 15–35, and 35–80 °C), with an accuracy of 0.5 °C. The system is totally controlled from a PC with custom-designed software, meaning that several slopes and constant temperatures can be preset. The results are presented in a visual and friendly environment.

The temperature control and slope generator system is composed of the following blocks:

1) temperature–time slope generation unit, where the cell current is modulated as a function of the initialization parameters
2) sensing and preprocessing unit, which acquires the plate temperature and converts it from analog to digital data (A/D) sending it to the control unit
3) the Peltier cell (P) attached to the plate that produces the transference of the thermal energy
4) the thermal exchange system, which is composed of a pump that forces distilled water through a hole in the aluminum heat sink attached to the cell, a tubular radiator, and a liquid container (inside the system).

A safety circuit, to check if the water level is normal, was implemented to protect both the cell and the pump. A schematic representation of the temperature control system is depicted in Figure 1.

Working Principle

The thermoelectric properties of a semiconductor cell [8] are used to obtain the temperature changes on the plate with the biological sample. This principle is also used in thermoelectric cooling or heating [9]. One face of the Peltier cell (P) is in contact with the plate AP and the another one with an aluminum hole heat sink (D1). If the face in contact with the aluminum hole heat sink is forced to remain at a constant temperature, driving a modulated current, on the cell, it will be possible to vary the temperature in the other face. From a thermodynamic point of view, it is necessary to get a very large mass on one face of the cell. To satisfy the above requirement and considering that the entire plate mass is of about 200 g, a cooling circuit of 1,700 cm^3 of completely sealed distilled water was used. A submerged pump forced the liquid through an aluminum hole heat sink, attached to the Peltier cell, reaching a liquid flow of 5,000 cm^3/min. To improve the thermal performance, a second hole helicoidally aluminum heat sink (D2) was connected before the liquid returns to the container. Under these conditions, the maximum temperature difference obtained between the two faces of the cell was 40 °C with an average current of 3 A, whenever the heat was efficiently removed from one face of the cell. The Peltier cell is from Supercool working at 40 W and 12–15-dc V.

It is important to note that the temperature range could be programmed from 2 to 80 °C. To initialize the system, the required parameters are initial and final temperatures as well as slope temperature (the design admitted slopes are 2, 1, 0.5, 0.33, and 0.25 °C/min). From these reference values, the system feeds the cell with a modulated direct current

and simultaneously measures the sample temperature in the plate every 2 s. When the initial temperature is reached, a sound alarm indicates that the system is ready; and then to perform the programmed slope, it is necessary to launch the slope program. This facility is needed by the operator to purge the sample chamber and/or equilibrate some biological samples before the experiments.

To generate the required temperature slope, the controller modulates the current pulsewidth to the cell [8], taking into account the evaluated differences between calculated (over a fixed time) and measured temperature values every 2 s. The temperature range is divided into three segments: 10–15, 15–35, and 35–80 °C. In the first range, the pulsewidth-modulated current circulates in one-way direction, reaching the programmed temperature by thermal equilibrium between the plate–cell–heat sink and the environment. In the second range, the current direction is inverted on each pulse, cooling and heating the cell, to reach the programmed temperature. In the third range, the current circulates again in one-way direction, but in inverse sense to the first stage, heating the cell. With this temperature control method, it is not necessary to have a constant room temperature to operate the system. The process described before generates a slope with a temperature deviation in the order of ±0.5 °C.

Figure 2 shows a simplified operation diagram system. The temperature of the biological sample is measured inserting a miniature K-type thermocouple in a little holder of the plate, just in close contact with the FTIR sample cell. The thermocouple signal is then amplified with "cold-correction" instrumentation [5]. A temperature reference sensor (National Instruments; see www.ni.com) was used to perform this correction. Thereafter, the thermocouple signal is digitalized with a 10 b A/D converter; a 0.1 °C precision over the 0–100 °C range was obtained. Ten samples were acquired per second.

The electronic circuit hardware was implemented with an 8-b microcontroller

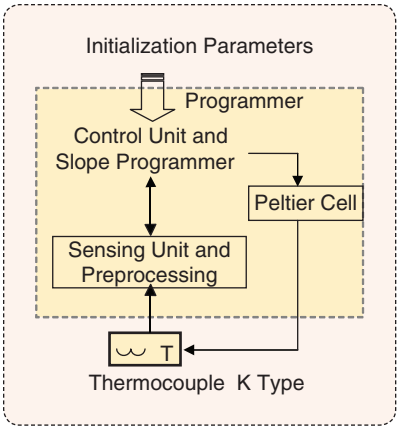

FIGURE 2 A simplified operation diagram system. The temperature of the biological sample is measured inserting a miniature K-type thermocouple in the little holder of the plate, in close contact with the FTIR sample cell.

16F87X (see www.microchip.com) family, with a clock frequency of 4 MHz. This integrated circuit has a programmable 14 kB flash memory, 256 EEPROM registers, 25 digitals programmable inputs/outputs, and eight analog inputs. The communication is implemented by I2C protocol and asynchronous USART. It is possible to program its operation using C language.

Two RS 232 ports were implemented in the controller to communicate with the user-interface control program in the PC and with the temperature register that evaluates the cell and ambient temperature.

The development of in vitro model systems of amyloid-fibril development from the soluble protein is of critical importance to gain knowledge about the physicochemical basis of amyloidosis.

The protection system was implemented by a floating sensor that activates two relays to open the current Peltier cell (the liquid pump power supply) and the sonic alert of failure. The electronic and electric devices are fed from a linear power supply with +5 V (regulated) and +12 V (unregulated), 50 W. The mechanical and electronic hardware system was mounted on an aluminum rigid box with 15 × 30 × 30 cm dimension (Figure 2).

The Acquisition Software

To configure all functional parameters and visualize the measurements and applications, the software Small Data Temp was developed in Visual Basic. This software is a friendly application showing the active keys, the programmed slope representation, and tables of the acquired values. The user can program 1) the initial and final temperatures as well as the temperature slope in the chosen time, 2) the data-acquisition intervals, and 3) port for devices and PC communication. Data could also be exported in an Excel format for further processing. Figure 3 shows the user interface.

Biological Calibration

FTIR has been extensively used for the detection and characterization of lipid-phase transitions in model and natural membranes. The methylene stretching vibration of the lipid chains gives two main bands, around 2,800 and 3,100 cm^{-1}, and is assigned to the antisymmetric (υ_{as} $[CH_2]$) and symmetric (υ_s $[CH_2]$) methylene stretching modes, respectively. The thermotropism of lipids is characterized by the shift of the wavenumber of these modes that have been shown to be sensitive to the lipids packing [10], [11]. For vesicles prepared with the zwitterionic phospholipid dipalmitoylphosphatidylcholine (DPPC), the melting temperature (T_m), obtained from different biophysical techniques, such as fluorescence spectroscopy, differential scanning calorimetry, and FTIR, is 41.5 ± 0.5 °C.

To test the operability and accuracy of our Peltier-based device, artificial lipid membranes (liposomes) of DPPC were placed in an FTIR sample cell, which was mounted in our thermostatic control and TST system. The slope of the thermal gradient was fixed at 1 °C/min, considering that the spectrum obtained with 64 scans (1 min of FTIR scanning) had the adequate signal/noise ratio. After these calibration and appropriated sample compartment purge, the DPPC liposomes were subjected to the temperature gradient. The heating range was selected from 10 to 80 °C. Before heating began, the system was equilibrated at 10 °C for 20 min to a complete water vapor removal from the sample chamber via a dry air purge. It is to emphasize that the Peltier-based device did not interfere with a correct purge.

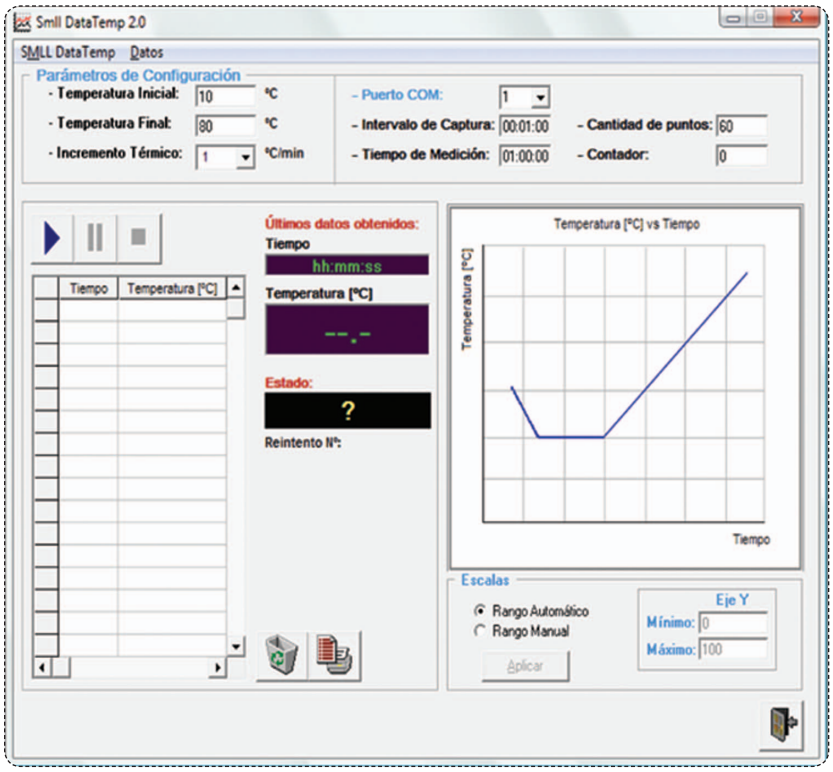

FIGURE 3 Small Data Temp user interface showing the active keys.

Figure 4 displays the evolution of the υ_s [CH_2] band metilen symmetric stretching vibration wavenumber of DPPC acyl chains as a function of temperature of four independent experiments. Considering the DPPC liposome thermal profiles, the T_m obtained was 41.5 ± 0.5 °C.

Amyloid Fibril Formation Assays

The development of in vitro model systems of amyloid-fibril development from the soluble protein is of critical importance to gain knowledge of the physicochemical basis of amyloidosis. In this article, we use the thermostatic control and TST system to improve the FTIR detection of temperature-induced amyloid-fibril formation.

The ability of bovine serum albumin (BSA) to form amyloidlike fibrils under certain experimental conditions has been recently described [12]. This fibrillation process occurs within minutes upon incubation of the protein in physiological pH at elevated temperatures. The infrared absorption bands related to the characteristic interchain β-sheets from the amyloid fibrils are located around 1,613 and 1,683 cm^{-1}. In this spectral region, the water vapor also absorbs and diminishes the signal/noise ratio. To decrease the water vapor noise, we incremented the number of scans/spectrum, and thus we had to change the heating slope. Our thermostatic control and TST system allows this kind of regulation according to the sample used.

The BSA was subjected to a thermal gradient with a heating rate of 0.5 °C/min, which allowed a data acquisition of 128 scans/spectrum. This fact allows spectra acquisition with better signal/noise ratio. It is important to note that after four independent experiments, the temperature in which the characteristic amyloid bands appear is perfectly reproducible. Moreover, the quality of the obtained spectra due to the increment of the signal/noise ratio allows the application of mathematical procedures to enhance spectral resolution as Fourier self-deconvolution and derivation (Figure 5).

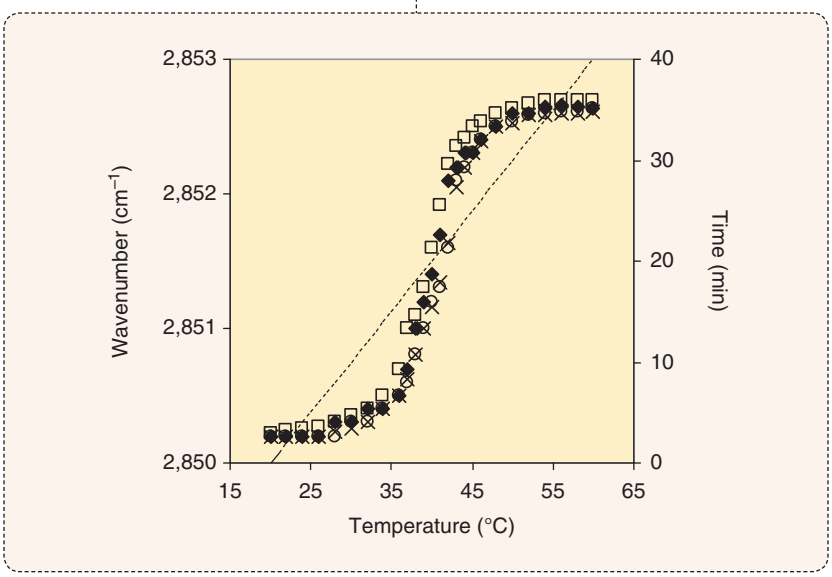

FIGURE 4 Temperature dependence of the wavenumber of symmetric methylene stretching vibration band of DPPC at pH 7.0 and 25 °C. The temperature slope is indicated by a dashed line.

Discussion

The development of a highly efficient and accurate Peltier-based system is reported

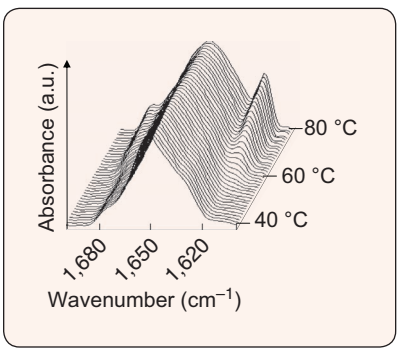

FIGURE 5 Stacked IR spectra of BSA recorded at regularly increasing temperature from 40 to 80 °C with a heating rate of 0.5 °C/min. The spectra have been deconvolved with a half width of 18 and $K = 1.75$.

to improve FTIR measurements. In this way, the phase-transition temperature of DPPC liposomes used herein as biological calibration is 41.5 ± 0.5 ºC, improving the previous values obtained by our group using the same spectrophotometer but with a thermostatic circulating water bath [13]. However, when the studies are focused in the spectral region where water vapor absorbs the infrared radiation, the water bath use turns critical. Unfortunately, the most important infrared spectral regions to study protein conformational changes are located between 1,700 and 1,500 cm^{-1}, where the water vapor has strong infrared absorption. In this way, the implementation of a system capable to change the sample temperature without generation of water vapor in the FTIR environment is a real contribution to improve the spectral quality and thus, the obtained results.

On the other hand, considering that the thermal-induced changes in biological systems, such as membranes and proteins, have a sigmoidal behavior, the accuracy of the temperature measurement in the sample itself is essential. In fact, lipid transition from gel to liquid-crystalline phase occurs in the tiny interval of 1.5–2 °C. Our Peltier-based system showed high accuracy with a small standard deviation (Figure 4).

On the basis of our quantitative experiments and qualitative observations, we are able to conclude that

- The device has a small size, and thus it does not interfere with the spectra acquisition since it was specifically designed to run inside the FTIR sample chamber.
- The device could efficiently operate without exchanging outside air, and thus the dryness obtained by the dry-air purge is not affected guarantying the quality of the spectra.
- The heating or cooling rate can be fine-tuned, and the reproducibility of the temperature slopes was high.
- The thermostatic control and TST system is a useful complement of FTIR spectrophotometer that is designed to regulate the thermal gradient according to the kind of biological sample.
- The accuracy and the small standard deviation obtained from the biological calibrations lead us to propose such thermostatic control and TST system as an excellent spectrophotometer accessory in biophysical studies.

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