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Photoacoustic monitoring of the absorption of isotonic saline solution by human mucus

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Abstract

Viscosity and elasticity are the fundamental rheologic properties of respiratory mucus, and are important determinants of transportability of mucus in the mucociliary system. One technique that permits to monitor indirectly the rheologic properties of any sample is the photoacoustic technique. Using that technique, the absorption of isotonic saline solution by human mucus was monitored. The mucus was obtained from 11 volunteers, divided into two groups: five volunteers presenting pneumology symptoms (group I) and six healthy volunteers (group II). The photoacoustic signal of the mucus absorbing the saline solution was monitored as function of time, with measurements being performed each 10 min, up to 120 min. The resulting curves were fitted to sigmoidal curves to simulate the evolution on time of the photoacoustic signal. A characteristic time for the half saturation of the absorption process was obtained. For group I the time obtained was 23.3 ± 5.3 min and for group II the time obtained was 55.0 ± 7.7 min, both means being significantly different (Student t-test, $p < 0.05$). This result supports the empirical practice of treating individuals presenting symptoms of airway obstruction with about 30 min of inhalations of isotonic saline solution vapor for the clearance of the airways.

Keywords: Photoacoustic techniques; Isotonic saline solution; Human mucus; Airway clearance

1. Introduction

The mucociliary apparatus of the tracheobronchial tree is one defense mechanism to protect the lung. It incorporates several defense strategies, for example, the surface liquids covering the airway epithelium trap impacted particles and ciliary action clears them from the tracheobronchial tree. In this way, mucociliary dysfunction can be defined as any defect in the ciliary and secretory component of the mucociliary apparatus that disrupts the normal physical, chemical, and biological defense functions of the airway epithelium [1].

Viscosity and elasticity are the fundamental rheologic properties of respiratory mucus, and are important determinants of transportability of mucus in the mucociliary system. It has been observed that the rheologic properties of mucus are often abnormal in patients with airway disease. In chronic upper and lower respiratory diseases, both the viscosity and the elasticity of respiratory mucus are much higher than the optimal values for mucociliary transport. Such abnormal rheologic properties could be a cause of decelerated mucociliary clearance in upper and lower respiratory diseases [2]. Because mucus transportability is best in a certain viscosity range, many drugs believed to optimize the rheologic properties of mucus have been evaluated for their beneficial effects on mucociliary clearance [1]. So it is important to have experimental techniques that permit the monitoring of the rheologic properties of mucus during the interaction with the analyzed drug. Typically the viscoelasticity of the nasal mucus is determined with an oscillating sphere magnetic rheometer [2]. Another technique that indirectly could monitor the rheologic properties of mucus is the photoacoustic technique.

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The photoacoustic (PA) signal results from the conversion of amplitude modulated electromagnetic energy to modulated thermal energy, through non-radiative decays in the absorbing material sample [3]. It depends on the thermal properties of the sample and of the base on which the sample rests, as the Rosencwaig–Gersho model shows [4]. Considering optically opaque and thermally thin samples, the PA signal has the following expression [4]:

$$S \approx \frac{(1-\mathrm{i})}{2a_\mathrm{g}} \left(\frac{\sqrt{2\alpha_\mathrm{b}}}{\sqrt{\omega} k_\mathrm{b}} \right) Y \tag{1}$$

where $\mathrm{i} = \sqrt{-1}$, a_g is the thermal diffusion coefficient of the gas inside the PA chamber, $\omega = 2\pi f$, f the chopping frequency of the incident beam light, α_b and k_b the thermal diffusivity and thermal conductivity of the base, respectively, and Y is a constant factor. In the PA chamber, the base corresponds to the material behind the absorber of the chopped light. Another thermal property is the thermal effusivity, defined as $e = k/\sqrt{\alpha}$. The thermal effusivity essentially measures the thermal impedance of the sample, or the sample's ability to exchange heat with the environment [5]. Using the definition of the thermal effusivity, Eq. (1) can be rewritten as follows:

$$S \approx \frac{(1-\mathrm{i})}{2ag} \left(\frac{\sqrt{2}}{\sqrt{\omega} e\mathrm{b}} \right) Y \tag{2}$$

Eq. (2) means that, in thermally thin and optically opaque materials, the PA signal is sensible to the thermal properties of the base. In this way, if the thermal properties of the base change, then the signal in the PA chamber also changes. This enables the PA technique to indirectly monitor different processes that change the structure, and consequently the thermal properties, of materials in the base position. Examples of this use of the PA measurements are the study of chemical reactions, as photopolymerization of dental resins [6], and also blood sedimentation [7], liquid drop evaporation [8] and transport of drugs through skin [9]. Different studies have established a relation between the thermal diffusivity and the viscosity of different systems, as corn masa flour [10] or wood pulp [11]. However, the relation established among both parameters is not unique and depends on the studied system, being for example a direct relation in reference [10] or an inverse relation in reference [11]. Nevertheless, as the photoacoustic signal depends on the thermal parameters of the mucus, its temporal evolution must reflect the temporal changes in its rheologic properties.

In this report, this technique is applied to study the absorption of isotonic saline solution (ISS) by human mucus. The main goal of this study is to monitor the ISS absorption process as a function of time, to estimate the absorption characteristic time in this process. Its importance is based on common therapies where patients with airways obstructed by mucus are indicated to breathe ISS vapor, in order to fluidize and expel the mucus more easily. Usually, prescription requires a 30 min-period of this inhalation therapy in a local hospital. The idea behind this therapy is that changes in the rheologic properties of the mucus must facilitate mucus elimination, promoting clearance of the airways.

2. Experiment

Initially, mucus from 20 healthy volunteers and 15 sick volunteers was obtained. The study was approved by the Ethics Review Committee of the Instituto de Pesquisa e Desenvolvimento at Universidade do Vale do Paraiba, São José dos Campos, São Paulo, Brazil, and the proper informed consent was obtained in writing from each volunteer before the mucus be collected. However, many samples were discarded due to the exclusion criteria adopted: excessive presence of saliva, presence of blood, higher dilution state and low quantity of mucus. After selection, the samples of mucus selected for analysis were obtained from 11 volunteers, being four females and seven males, with ages around 30 ± 5 years. From this group, five presented symptoms of pneumonia and were recovering at the Santa Casa Hospital in São José dos Campos, São Paulo, Brazil. The other six volunteers were healthy adults from the Instituto de Pesquisa e Desenvolvimento at Universidade do Vale do Paraíba. For analysis, volunteers were distributed into two groups: group I was formed by the five volunteers with pneumonia symptoms and group II was formed by the six healthy volunteers. To collect the mucus, we followed the protocol stated by Bossi [12]. The collected mucus was stored in Eppendorf tubes filled with vaseline oil to avoid dehydration. The samples were stored at −45 °C until experimental use. After unfreezing, each mucus sample was briefly submersed in petroleum to eliminate the vaseline oil [13]. The PA setup comprised a Tungsten arc lamp (150 W), a mechanical chopper, lenses, a PA cell and a two-phase lock-in amplifier. Light was chopped at 23 Hz. As Fig. 1 shows, the PA cell had two faces, one of them closed with a glass window and the other closed with an aluminum foil (25 μm thick). The PA chamber had a diame-

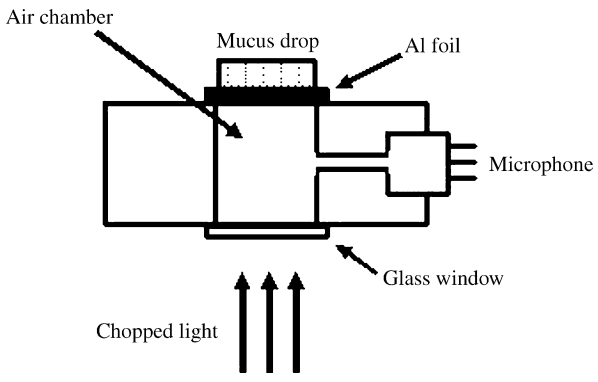

Fig. 1. Diagram of the photoacoustic cell used in this study. Chopped light impinges first in the glass window that closes one face of the cell and later in the aluminum foil that closes the other face. The mucus sample rests over the aluminum foil.

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ter of 0.8 cm, with a depth of 1 cm, accounting for a volume of 0.5 cm^3. The light was incident from the front, impinging first on the glass window. The power of the incident light was 100 mW, distributed homogeneously in the whole surface of the aluminum foil (about 0.5 cm^2). The aluminum foil absorbed the white light generating the PA signal. As the absorber used in this experiment (Al foil) is optically opaque and thermally thin at 23 Hz, the corresponding PA signal will depend on the thermal properties of the material over the aluminum foil; if the thermal properties of this base change, then the amplitude and phase of the signal will also change. An electret microphone detected the sound generated in the PA chamber, and its voltage was directed to the lock-in amplifier, that was interfaced to a computer to collect the experimental data. A program controlled the chopper and the lock-in.

Measurements were done as follows: about 0.1 mL of mucus was put on the aluminum base and the PA signal was monitored for 1 min. This measurement was repeated after 10 min, and 10 min later too. These initial measurements were done to give information about the level of the PA signal before the interaction with isotonic saline solution. After 20 min, the mucus was taken from the aluminum base and introduced into an Eppendorf tube filled with 1.5 mL of ISS, where it was kept for 10 min. After this time another measurement was done, putting the mucus sample on the PA chamber and being put inside the ISS Eppendorf again after the measurement. This procedure was repeated 10 times, for up to 2 h of total elapsed time.

At every measurement time 50 repetitions were done at intervals of 3 s, lasting 2.5 min for the measurement of each experimental data. This was done in this way to monitor the stability of the system. So, the error showed in Fig. 2 corresponds with the standard deviation of the 50 measurements done.

The experimental data obtained were analyzed using the analysis tools of the software Microcal Origin 6.0.

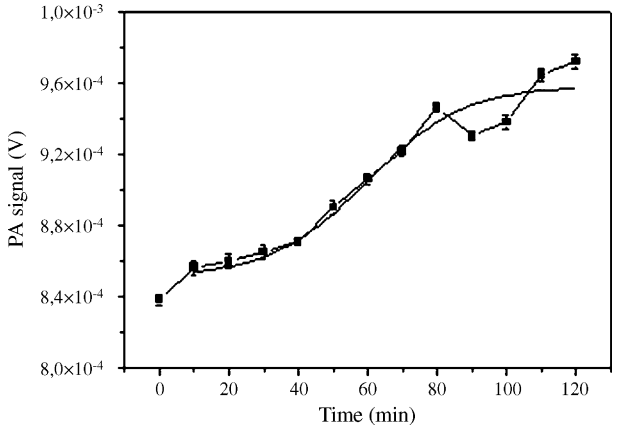

Fig. 2. PA signal as a function of time of ISS absorption by the mucus. The square represent the mean value and the bar in every square represents the standard deviation of 50 measurements. Continuous line represents the data fit to Eq. (3).

3. Results and discussion

Fig. 2 shows an example of the obtained PA signal as a function of time for a mucus sample. This curve corresponds with a mucus from group II, but by no means it is representative of this group. As it can be seen, the signal follows a monotonic growth with time. The experimental data were fit to the sigmoidal function:

$$S(t) = A_1 + \frac{A_2 - A_1}{1 + e^{(t-t_0/\Delta t)}} \quad (3)$$

where A_1 is the basal level, A_2 the saturation level, t_0 the time to reach the maximum rate of change in the process, and Δt is the time interval during which the saturation of the ISS absorption process takes place. For the study done here, the most interesting parameters are t_0 and Δt because they characterize the absorption time of the isotonic saline solution by the mucus. In this process, t_0 is related to the half-saturation time of the solubilization process. Parameters A_1 and A_2 are related to the physical characteristics of the mucus, such as quantity and density, because these variables determine the intensity of the signal. As the aim of the present study is to analyze the kinetics of ISS absorption process, the parameters A_1 and A_2 were ignored. Table 1 shows the results for the two parameters t_0 and Δt for the studied groups. It can be seen that samples from group I show lower values for these parameters than those presented by group II. A Student's t-test was carried out to evaluate if the mean values of t_0 and Δt are significantly different between groups I and II. For both parameters, average values were significantly different ($p < 0.05$, Table 1). The power of the Student's t-test done with the groups for t_0 and Δt was calculated [14] and the probability β of committing a type II error was lower than 0.01. This result shows that the difference observed among the calculated mean values can be sustained with the number of measurements done in each group. Fig. 3 shows the Δt versus t_0 values for volunteers in each group. As can be seen, each group is well clustered in different regions of the plot of Δt versus t_0. This means that each group of mucus sample has different absorption kinetics for the isotonic saline solution. In other words, the absorption kinetics must be related to the pathology associated with the mucus. Also, it can be observed that the adjusted data in every group show a high dispersion, and for this reason the Fig. 2 it is not representative for the group II. In the Fig. 3 and Table 1 one can see that the mucus

Table 1
Mean values and standard deviations for parameters t_0 and Δt in both studied groups

	Group I	Group II
N	5	6
t_0	23.3 ± 5.3 min	55 ± 7.7 min
Δt	4.5 ± 1.9 min	16.6 ± 3.4 min

N corresponds with the number of individuals. The t-Student test between parameters in both groups showed that mean values are significantly different ($p < 0.05$).

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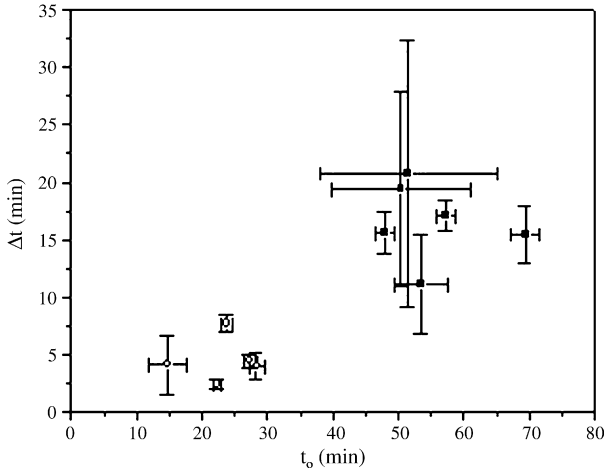

Fig. 3. Graph of Δt vs. t_0 for clustering analysis. Black squares refer to group II data and open circles, to group I data. Bars in each symbol correspond with the fitting error of Eq. (3) fitted to experimental curves.

from volunteers in group I presents a half saturation time of about 25 min, close to the time period often employed in therapeutical treatments of nebulization in patients with pneumonia.

The idea behind the absorption of the ISS is to solubilize the mucus, which is then eliminated more easily from the airways. However, when the mucus is solubilized, its rheologic properties change. In this way, a relation between the PA signal as a function of time and the variation of rheological properties with time can be seen. Indirectly, the curve in Fig. 2 monitors the rheologic properties of the mucus during ISS absorption as mentioned in the introduction.

4. Conclusion

This study has shown that mucus from individuals with different health status present different characteristic times for absorption of isotonic saline solution. The results obtained show for the first time that the empirical practice in the treatment of individuals with symptoms of airway obstruction is correct. More studies must be carried out to analyze the effect of mucolitical drugs in mucus from different sickness pathologies.

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