METHODS

Use of Infrared Spectroscopy for Analesis of Expired Air Condensates (A New Method)

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A new method for analyzing noninvasively procured biological materials - expired air condensates - using infrared spectroscopy is described. The proposed method, which involves smoothing out of the condensate spectra and subtraction of the interpolated water spectrum, may be employed in basic research and in developing diagnostic procedures based on quantitative estimation of the organic condensate component.

Key Words: *infrared spectroscopy; expired air condensate*

Recent years have witnessed mounting interest in the application of infrared (IR) spectroscopy for the analysis of biological materials with a view to their further use in the diagnosis of various diseases or for solving problems that require separation of the groups under study [2,4,9,10]. The main factor limiting the applicability of this method is the need to work in aqueous media, where the intensive absorption of water in the IR region prevents the detection of organic substances present in minor quantities. The study reported here was undertaken to devise a method for IR analysis of noninvasively obtained biological material, namely expired air condensate (EAC) specimens with a view to the subsequent development of diagnostic procedures based on quantitative estimation of the EAC's organic component with statistical processing of the IR spectra obtained.

MATERIALS AND METHODS

All measurements were made with a Specord M82 IR spectrophotometer (Karl Zeiss) hooked up to an IBM PC/XT. A drop of EAC (40 μl) from each specimen was compressed between two sapphire glasses 0.8 mm in thickness and 15 mm in diameter (giving a window of 4000-1700 cm^{-1}) and placed in a standard cuvette holder, so that the EAC layer was approximately 0.015 mm thick. To rule out evaporation of this capillary layer, the ends of the sapphire glasses were coated with a silicone lubricant. All EAC specimens were stored frozen at -10°C, a temperature at which the composition of organic compounds in an aqueous solution has been shown to cease changing after sev-

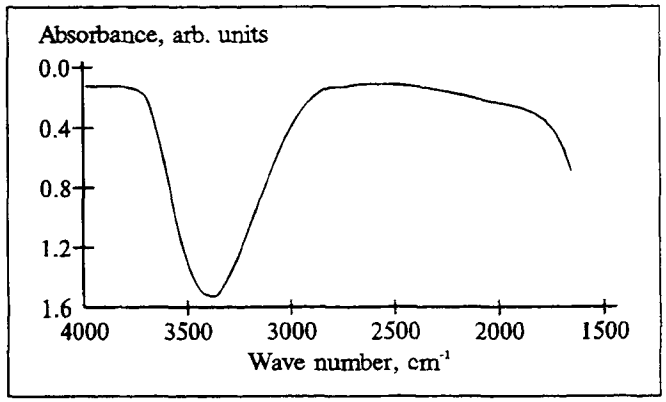

Fig. 1. IR spectrum of expired air condensate from a healthy subject.

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eral weeks of storage [7]. They were thawed out at room temperature before analysis.

Spectra were recorded using a two-beam scheme, without a reference sample, in the 4000-1700 cm^{-1} area with a step of 1 cm. The integration time was 1 sec and the total recording time was 1 h 40 min; an isoenergetic slit (40%) was used. The mean spectral absorbance was 0.49±0.15, i.e., it lay within the optimal range of absorbance values.

RESULTS

A total of 20 EAC specimens from healthy subjects were analyzed. A typical EAC spectrum for a healthy human being is shown in Fig. 1. This spectrum, digitized using a 1 cm^{-1} grid, was smoothed out 5 times for three points (Fig. 2, *a* and *b*) and extended 300-fold. Since the thickness of the absorbing layer was not normalized, normalization was done for the area under the spectrum, i.e., for the quantity of water. The absorption spectrum of the solvent (water) was then interpolated and subtracted from that of the sample (Fig. 2, *c-e*). The interpolation was based on the assumption that the specimen's spectrum comprised a superposition of powerful and broad water absorption bands and narrower and much less intensive (approximately by two orders of magnitude) absorption bands of dissolved substances. Because of this, we chose as interpolation nodes the points at which the curvature of absorption as a function of the wave number was minimal [9]. The spectra of the remainder (Fig. 2, *e*) will apparently correspond to that of substances dissolved in the condensate.

For the subsequent analysis of intergroup differences, samples of 4 to 15 spectra were formed and compared for absorbances in the respective peaks using the nonparametric Wilcoxon-Mann-Whitney test [1]. All calculations were performed with software we devised for quantifying absorbance values and detecting significant differences among the peaks in the samples. After these differences had been revealed, the particular peaks were identified by the use of appropriate correlation tables [5].

The present study has thus enabled us to propose a new method for analyzing the organic component of EAC specimens using IR spectroscopy. The method may find application both in basic research and in diagnostic practice. The simplest use to which it can be put may be a phenom-

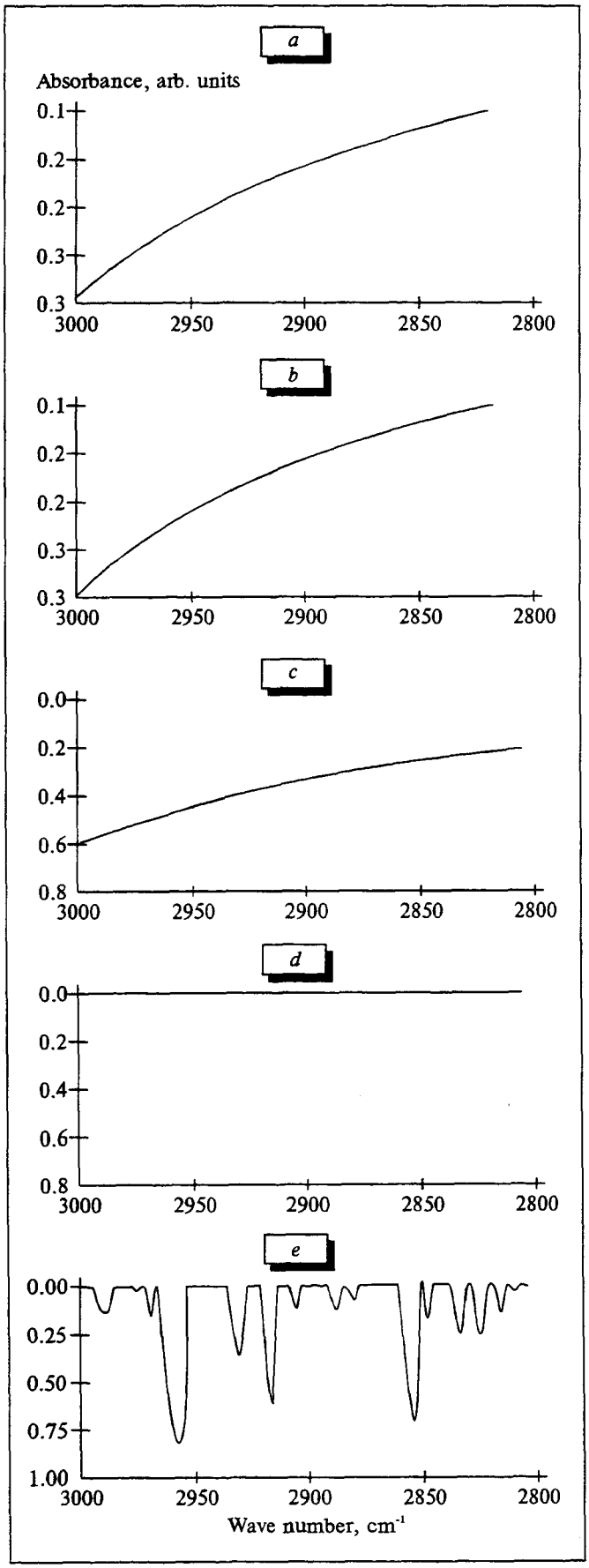

Fig. 2. Stages in the processing of a spectrum fragment. *a*) fragment of unprocessed spectrum; *b*) fragment smoothed out for three points; *c*) interpolated spectrum of water; *d*) spectrum of the remainder; *e*) spectrum of the remainder extended 300-fold.

enological analysis of EAC specimens, while the identification of distinct peaks may contribute to a better understanding of the mechanisms by which pathological processes arise. In forthcoming publications we will present the results obtained for particular groups of subjects.

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