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# The Potential of Fourier Transform Infrared Spectroscopy for the Analysis of Confectionery Products

Peter S. Belton, Alfred M. Saffa, Reginald H. Wilson

AFRC Institute of Food Research, Norwich Laboratory,
Colney Lane, Norwich, Norfolk NR4 7UA, UK

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Arthur D. Ince

Cadbury Ltd, Bournville, Birmingham B30 2LU, UK

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*ABSTRACT*

*The potential of Fourier transform infrared spectroscopy for the analysis of confectionery products was demonstrated using chocolate and cocoa liquors as representative materials. Both photoacoustic and attenuated total reflectance detection methods were used. Both methods gave well resolved signals which could be assigned to fat, protein and sugar. It was found that photoacoustic spectroscopy was the best method for fat analysis. However, attenuated total reflectance techniques were shown to be useful for investigating phase changes occurring in the lipid components during both cooling and storage of chocolate.*

## INTRODUCTION

Near infrared reflectance (NIR) spectroscopy has gained wide acceptance in the food industry as an analytical technique. This is despite the need for large calibration sets and correlation methods. So far, the mid infrared region



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(4400–400 $cm^{-1}$) has not been exploited to any great extent. This has been due largely to the inability in the past to obtain reasonable quality spectra from intact samples of the type encountered in the industry. These samples can exist in a wide range of physical states or in aqueous media. The advent of Fourier transform infrared (FTIR) spectroscopy, coupled with new sampling methods, now makes the acquisition of high quality spectra from these materials possible. The mid-infrared region has definite advantages when compared with the near infrared, particularly in that bands can be assigned to specific chemical entities. Furthermore, these bands are usually much better resolved and hence less sophisticated quantitative methods are required and, generally, less calibration work is needed. Of course, very complex mixtures may still need correlation methods so these are not excluded. The disadvantage of FTIR has been the relatively high capital cost of instrumentation. However, the continued downward trend in costs and the possibility of multiplexing a number of optical heads to a single computer makes this form of spectroscopy increasingly competitive. Confectionery products, and chocolate in particular, represent a real challenge to any optical technique. Chocolate is dark and opaque, and on melting it forms a viscous liquid. It is thus very unsuitable for conventional transmission techniques. In this paper we investigate Ftir as a method for fat analysis and also to give an insight into the other sorts of information which might be obtained. The sampling methods chosen are photoacoustic spectroscopy (PAS) and attenuated total reflectance (ATR). Previous work has shown that these are two of the potentially most useful of the new sampling methods for intractable materials. The relevant spectroscopic principles are discussed in detail elsewhere (Griffiths, 1975; Harrick, 1967; Rosencwaig, 1980) and only a brief description is now given to make the experimental section more intelligible.

In ATR (Harrick, 1967) the infrared light is passed into a prismatic crystal of high refractive index at such an angle that it is totally internally reflected at the crystal surface. The light is then reflected in a zig-zag fashion until it emerges from the other end of the crystal and hence passes to the detector. At each internal reflection there is coupling between the oscillating electric fields in the crystal and the medium with which it is in contact. This coupling leads to an attenuation of the beam equivalent to an absorbance over a very short pathlength. The sample therefore only has to transmit infrared light over a short pathlength but must produce a good optical contact. Thus, ATR is ideal for soft, compressible materials or molten samples that can easily be spread onto the crystal.

In PAS (Rosencwaig, 1980) the sample is held in a sealed cell of constant volume. Non-radiative de-excitation occurs in the sample following absorption of light and in the presence of a modulated infrared beam this

leads to the generation of a thermal wave. The thermal wave causes a periodic variation in the pressure of the gas in the cell that is detected with a microphone. PAS is reasonably insensitive to sample morphology and is useful for most types of solid and some liquid samples, except those with a high water content. The latter do not produce good PAS spectra as water vapour and gases generally produce a large photoacoustic effect which may seriously distort the spectrum.

## EXPERIMENTAL

Samples were of moulded, solidified cocoa liquors (cocoa solids and cocoa butter) and chocolate (cocoa liquor, sugar, milk fat and milk solids). These samples had fat contents in the range 28·9 to 55·3% as measured by the IOCC (1963) method, involving Soxhlet extraction after an acid digestion. Ageing studies were carried out on a variety of commercial chocolate samples before and after storage. Two methods of sample preparation were used, depending upon the sampling optics used. The simplest was to break off a piece of chocolate or solidified cocoa liquor and clamp it to the ATR crystal face. The second method involved applying the samples in a molten state. The samples were melted in a beaker standing in water at 80°C. The molten material was then spread onto the ATR plate. Both methods produced ATR spectra of high quality. PAS samples were all introduced to the cell in the molten state.

All FTIR measurements were carried out on a Digilab FTS60 spectrometer equipped with a TGS detector and operating at either 4, 16 or 32 $cm^{-1}$ resolution and 0·32 cm/s mirror velocity. 256 or 64 interferograms were co-added before Fourier transformation. Triangular apodization was employed.

PAS measurements at 16 $cm^{-1}$ (256 scans) or 32 $cm^{-1}$ (64 scans) resolution were made using a Digilab PAS cell fitted with a $CaF_2$ window. Carbon black was used a reference material and spectra were presented in normalised photoacoustic intensity units. It was found that best results were obtained if the samples were left for about 5 min before spectral acquisition. The acquisition time was approximately a further 5 min at 16 $cm^{-1}$ resolution and 1 min at 32 $cm^{-1}$ resolution. The 5 min delay is probably associated with thermal equilibration of the cell.

Two types of ATR technique were employed. For ageing studies a Spectra-Tech continuously variable ATR accessory was used with a Ge parallelogram crystal (50 × 20 × 3 mm, 45°) and incident angle set to 45°. Pieces of the sample were clamped to the crystal with the minimum pressure

required to produce good optical contact. For ageing studies the direct clamping to the crystal meant that no heating of the sample was necessary. Therefore, important information regarding lipid phase changes during storage was not destroyed as was the case with the samples applied molten to the horizontal ATR or the PAS. The second type of ATR accessory used was a Spectra-Tech horizontal ATR attachment fitted with a ZnSe crystal. Samples were introduced to the crystal in a molten state and allowed to cool. Spectra were obtained at various times during cooling and for these experiments only 64 scans were used. For all ATR experiments the resolution was $4\,cm^{-1}$ and all sample single beam spectra were ratioed to a single beam spectrum of the accessory without any sample. Spectra were presented in absorbance units.

For quantitative work, comparison of ATR and PAS showed that PAS was preferable. In the ATR method the intensity of the spectrum depends on both the intrinsic absorptivity of the material and the quality of optical contact. This, in turn, depends on both the surface roughness of the material and the pressure applied to the sample. Generally, these cannot be very well controlled. However, for materials of a similar physical state, such as those used in this work, the crucial parameters are applied pressure and crystal coverage. The design of the ATR accessory used in this work was such that neither of these two factors could be controlled and hence quantitative ATR was not possible. In PAS, the critical factor, apart from intrinsic absorptivity, is the acoustic contact between the sample and the transmitting gas. For samples of constant particle size or liquids this is constant. In the PAS cell used here the radiant energy flux was sufficient to keep the surface of the samples molten. This ensured that acoustic contact remained constant. Since PAS is a surface sensitive technique only the molten part of the sample generated a signal; hence, no information about solid fat phases was obtained.

In FTIR, signal to noise ratios, for a constant number of scans, are reduced when resolution is increased. Thus, for PAS, which is intrinsically a low signal to noise technique, low resolution ($16\,cm^{-1}$ or $32\,cm^{-1}$) conditions were used to minimise the number of scans required to produce an acceptable signal. Generally, bands in PAS spectra tends to be broader than in transmission spectra so that decreased resolution does not degrade the intrinsic spectral resolution, although, compared to other methods, PAS may result in some loss of spectral detail. In the spectra considered here there was some loss of detail in the sugar bands. ATR, by contrast, is a method with intrinsically higher S/N ratios and band widths similar to those observed in transmission spectra; thus higher resolution ($4\,cm^{-1}$) acquisition conditions allowed well resolved spectra to be obtained in only 64 scans. Both lipid and sugar bands were well resolved by ATR.

## RESULTS AND DISCUSSION

Typical spectra using ATR and PAS are shown in Fig. 1. In both cases the sample was introduced to the sampling accessory in the molten state. In the PAS spectrum a number of bands arising from fat can be seen, with the strongest being at 1744 $cm^{-1}$ arising from C=O (ester). Other fat related bands occur at 1477–1400 $cm^{-1}$ (C—H bend), 1240 $cm^{-1}$ and 1195–1129 $cm^{-1}$ (C—O stretching). Bands arising from the other components of the chocolate: protein (1650 and 1540 $cm^{-1}$) and sugars (a number in the region 1128–952 $cm^{-1}$) can also be seen. The resolution in these spectra is such that, in principle, all components could be quantified simultaneously given suitable standards. However, for the purposes of this work, a detailed study was limited to the fat content.

Fat analysis was carried out by using the 1744 $cm^{-1}$ peak in the PAS spectrum. This band is the most intense lipid band and has the further advantage of being well separated from the other bands. Quantitative PAS results are presented as the reciprocal of the PAS intensity at 1744 $cm^{-1}$ versus the reciprocal of the percentage fat in the sample, following the

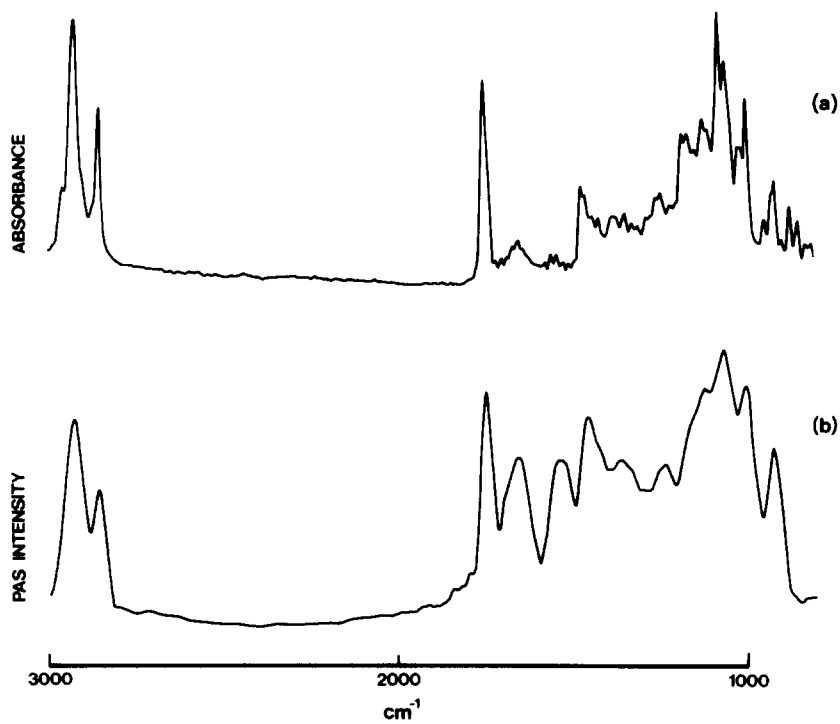


**Fig. 1.** Typical chocolate spectra obtained from samples applied in molten state using (a) ATR at 4 $cm^{-1}$ resolution and (b) PAS at 16 $cm^{-1}$ resolution.

method described by Belton and Tanner (1983). The calibration plots are shown in Fig. 2. A linear relationship was obtained at both resolutions employed. At 16 $cm^{-1}$ resolution, the slope was 0·646, intercept 0·02 and correlation coefficient 0·98. At 32 $cm^{-1}$ resolution, the slope was 0·855 with identical intercept and correlation coefficient. The variation in slope was expected as the change to lower resolution leads to peak height reduction because of peak broadening but the overall band area is maintained. The correlation coefficient shows that 32 $cm^{-1}$ resolution and 64 scans co-added are adequate to determine fat contents in the system, although further method refinement would be required to produce the required practical accuracy.

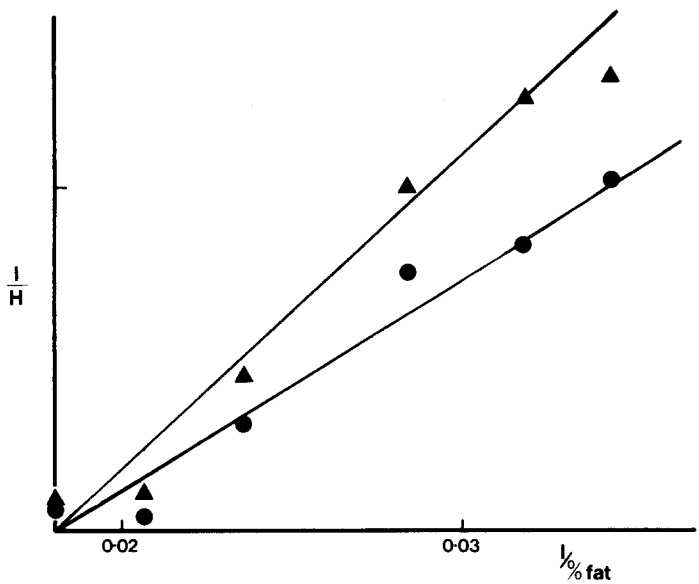


**Fig. 2.** Plots of the reciprocal of the PAS intensity ($1/H$) at 1744 $cm^{-1}$ versus the reciprocal of the % fat in chocolate samples. (Vertical scale 0·01 units $div^{-1}$.) ●, 16 $cm^{-1}$ resolution. ▲, 32 $cm^{-1}$ resolution.

During early investigations it was clear that the bandshape of the lipid peak of certain samples changed dramatically after application to an ATR plate in the molten state and subsequent cooling. These effects are not seen in PAS because of the sample heating effects. Therefore, an investigation was carried out into these changes using time resolved Ftir. The results from this experiment are shown in Fig. 3 for the cooling, over a period of 2 h, of cocoa liquor and chocolate samples. In this Figure only the C=O ester band is shown although all of the lipid peaks were affected to some extent. The changes seen in these spectra are related to phase changes during the cooling

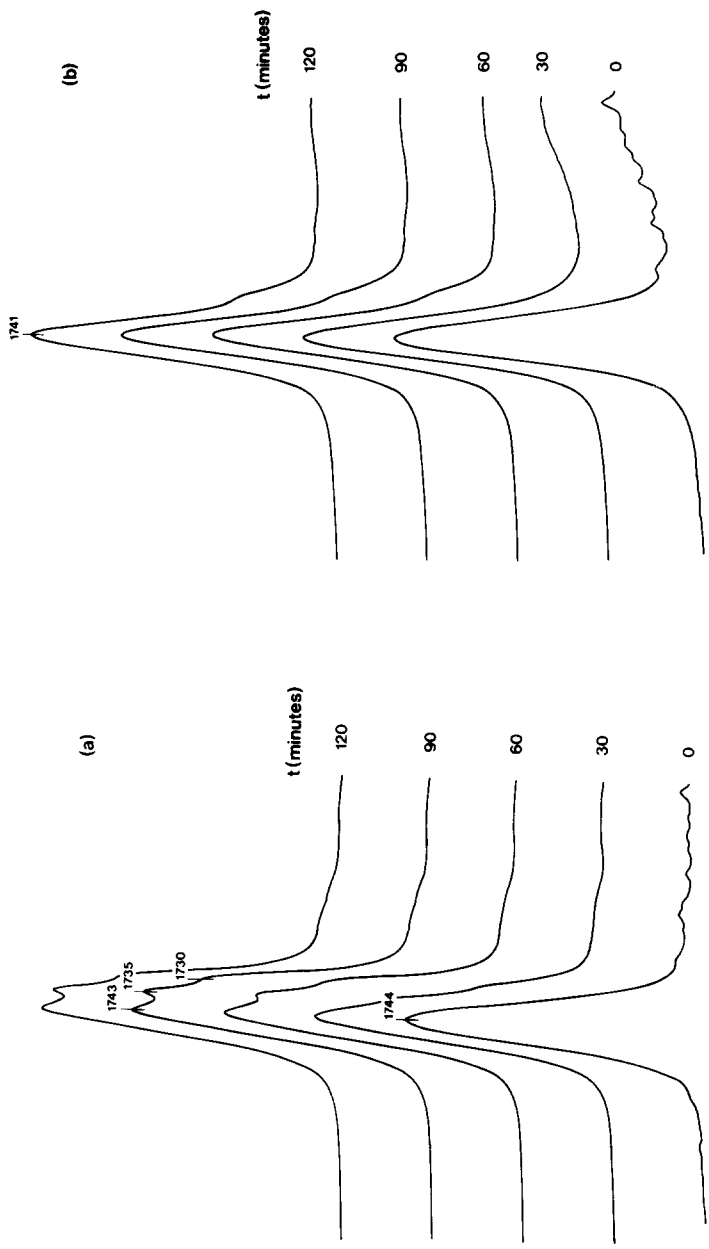


**Fig. 3.** Change in lipid bandshape on cooling for (a) cocoa liquor, 55·5% fat and (b) chocolate, 28·9% fat. Numbers with each spectrum refer to time ($t$) in min after application to ATR plate.

of the samples and are fully reversible. Clearly, there is a great difference between the two samples in regard to the extent of phase separation. This observation is important from an analytical point of view since any quantitative determinations may be affected by the extent of cooling and the degree of phase separations. It is suggested, therefore, that analysis of these materials is carried out in the molten state.

Chocolate samples applied to an ATR crystal without prior heating retain phase information and the spectra of a fresh and stale chocolate show differences in lipid bandshape (Fig. 4). It appears that phase separation is a major process occurring during the storage of this product. This opens up the possibility that ATR may be used to monitor such changes in stored products.

In conclusion, PAS is the best method for quantitative analysis of fat, at the moment. However, if some attention is paid to ATR cell design then this technique should have great potential. The ideal type of ATR cell may be a

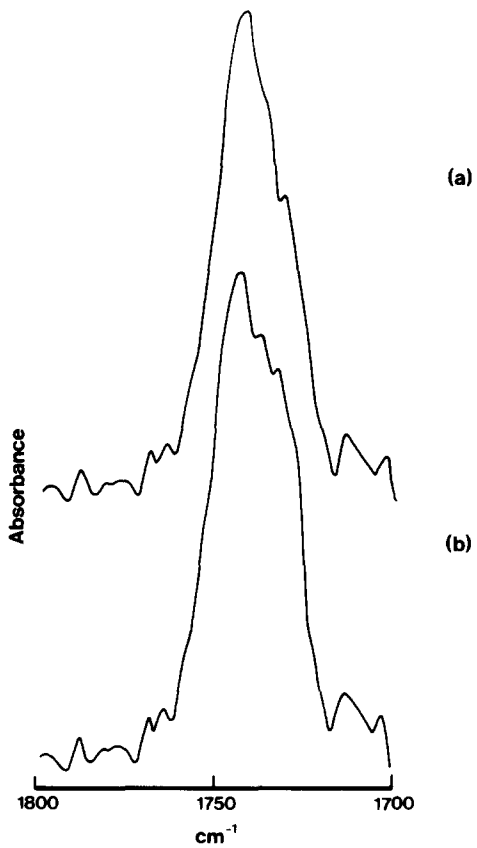


**Fig. 4.** Differences in lipid 1744 $cm^{-1}$ bandshape between (a) fresh and (b) stale chocolate samples by ATR.

single reflection type with a heated, fixed volume reservoir on top of a horizontally mounted crystal. The constant volume cell would ensure reproducible crystal coverage and, for similar samples, would ensure consistent applied pressure to the crystal. The inherently better signal to noise ratio of ATR could then be exploited to the full. Furthermore, the 5 min equilibration period required with PAS would be eliminated. Typical acquisition time would then be about 1 min in total as opposed to about 6–10 min per sample as currently required by PAS. Even with existing cell designs the ability to acquire high resolution spectra with good S/N by ATR with minimal sample preparation makes it the best method for qualitative studies. This is particularly important in such systems as those studied here where sample heating or grinding is likely to destroy the information content. The high resolution available allows the extraction of the maximum amount of such information from these systems.

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