Food Chemistry **16** (1985) 115–131

Application of Infrared Reflectance Spectroscopy to the Analysis of Milk and Dairy Products

John F. Kennedy, Charles A. White

Research Laboratory for the Chemistry of Bioactive Carbohydrates and Proteins,
Department of Chemistry, University of Birmingham,
PO Box 363, Birmingham B15 2TT, Great Britain

&

Anthony J. Browne

Buck Scientific Ltd, Litchborough Rural Industrial Estate,
Towcester, Northants NN12 8JB, Great Britain

(Received: 21 August, 1984)

ABSTRACT

A method for the rapid, simultaneous determination of protein, fat, carbohydrate, non-protein nitrogen and total solids in milk and liquid dairy products by infrared reflectance spectroscopy is presented. The method is calibrated against known standards obtained by conventional, time-consuming wet chemical methods but, once calibrated, can be operated routinely by non-skilled personnel. The method is sensitive and accurate down to 0·5% w/v and can be applied to most liquid samples without any need for prior preparation.

INTRODUCTION

The major components of milk and dairy products for which accurate quantitation is required include protein, fat, carbohydrate and non-protein nitrogen. The determination of such components in mixed

Food Chemistry 0308-8146/85/$03·30

solutions presents many difficulties to the food industry. Each component has to be determined specifically by the traditional methods of analysis (such as the Kjeldahl method for protein (Lillevik, 1970), the Rose–Gottlieb method for fat (Hubbard *et al.*, 1977) and the Lane and Eynon (1923) or enzyme (Bahl, 1972) methods for lactose which are time-consuming, subject to interference and require highly skilled laboratory personnel and highly-equipped laboratories.

With ever increasing legislation covering the control of food products and steadily rising raw material costs, food processing industries must become more cost-effective to remain competitive (Maes, 1979). Major savings can be made in analytical services if analysis times can be reduced to allow a more rapid response to changes in composition, etc., which affect product specifications, simple techniques can be adopted which do not require highly skilled laboratory personnel or high cost laboratories and versatile equipment can be employed which does not become redundant with changes in product manufacture. Infrared reflectance spectroscopy provides an ideal technique to satisfy the above criteria whilst allowing the simultaneous determination of a number of components in admixture. Since such spectroscopic methods do not require the use of any chemicals, the analytical system can be sited adjacent to the production process which further reduces delays in obtaining analytical results.

In traditional (transmission) infrared analysis the sample is confined between plates of infrared-transparent material and infrared energy is beamed through these 'windows'. The amount of energy which emerges at the various wavelengths is measured and compared with the original energy. The amount of energy which passes through the sample is dependent upon the concentration and absorptivity of the sample and the pathlength through the sample. For highly absorbing samples (most liquid streams), the pathlength must be quite short. This presents a problem, particularly for process applications, where a constantly flowing stream must be forced between two closely spaced plates.

The Multiple Internal Reflection (MIR) technique has been known for approximately 20 years under various names including Attenuated Total Reflection (ATR), Frustrated Multiple Internal Reflection (FMIR) and Frustrated Total Internal Reflection (FTIR).

In an MIR cell, the sample surrounds an optical crystal. This crystal may be one of several materials which will be described later. Infrared energy is beamed at an angle into one end of the crystal. As shown in

Fig. 1, this beam is reflected back and forth between the surfaces of the crystal in contact with the sample. If the sample does not absorb any infrared energy, then all the energy is totally reflected within the crystal but if the sample does absorb energy, then some is lost at each point of reflection as the beam slightly penetrates the sample. Energy emerging from the crystal is measured and compared with the incident energy to determine the total infrared energy which has been absorbed.

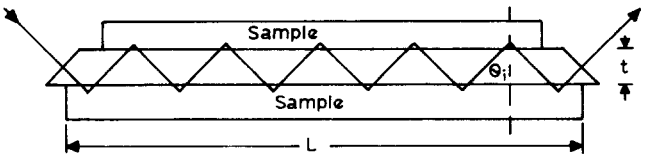

Fig. 1. The multiple internal reflection (MIR) effect (L = length of crystal, t = thickness of crystal and θ_i = angle of incidence at crystal/sample interface).

When the sample is highly absorptive, the MIR cell can be used for analysis where a transmission cell, even of very short pathlength, would be impractical. Figure 2 shows an example of the difference between a transmission cell and the MIR cell when used on a water sample. Although there are some wavelengths at which the water absorbs 100 % of the energy even with the MIR cell, there are other wavelengths at which it is possible to obtain a quantitative analysis.

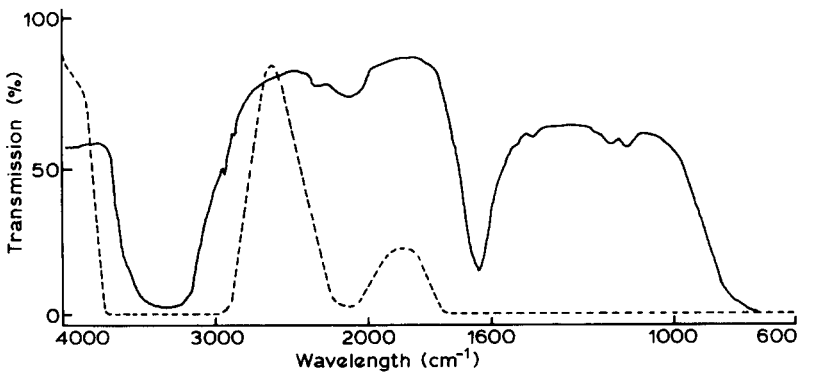

Fig. 2. Difference in transmission levels recorded with wavelength for the analysis of water using ····· calcium fluoride transmission cell (0·1 mm pathlength) and —— zinc selenide MIR crystal (50 mm × 2 mm thick, $\theta_i = 60°$).

The MIR cell has these advantages over a standard transmission cell:

(a) Wide range. It can be used with infrared analysers to provide quantitative data for concentrations between 0·5% and 100%, depending upon the component of interest.
(b) Fast response. Because the MIR cell does not compel the sample stream to flow through narrow passages to obtain a short pathlength, as in a transmission cell, a considerably higher flow rate is possible.
(c) It can be used for the analysis of aqueous solutions. Because water is highly absorptive at many infrared wavelengths, such solutions present many problems for infrared analysis when using standard transmission cells. MIR, as stated previously, can be used because of the very small penetration of the infrared beam into the sample as it traverses through the crystal.

There are a number of factors which determine the amount of energy absorbed by the sample at specific wavelengths. First, in order to be well away from the critical angle (the limiting angle of total internal reflection) and to ensure multiple internal reflections, it is desirable that the refractive index of the crystal is considerably greater than that of the sample. Since most organic compounds have a refractive index of about 1·5, ideally the refractive index of the crystal should be above 2·0. Table 1 shows the refractive indices and available ranges of wavelengths for several crystal materials which can be used in infrared work.

A second factor affecting energy absorption is the number of internal reflections which occur within the crystal (see Fig. 1). This can be determined approximately from the following:

$$R = \frac{L}{t} \cot \theta_i$$

where: R = number of reflections; L = length of the crystal; t = thickness of the crystal and θ_i = angle of incidence at crystal/sample interface.

As energy is absorbed at each reflection, the more reflections there are, the more infrared energy is absorbed and the greater the sensitivity of the system. As shown in Fig. 3, the number of reflections can be varied by changing the angle of incidence for a given application and therefore sensitivity can be increased by increasing the angle of incidence. In MIR the depth of penetration of the infrared radiation into the sample increases linearly with the wavelength (all other factors remaining

TABLE 1
Crystal Materials Used in MIR Cells

Crystal material	*Operating wavelength range (10^{-6} m)*	*Refractive index*
Sapphire (Al_2O_3)	0·22–4·5	1·7
Zinc selenide (ZnSe)	1·0–18·0	2·4
Silicon (Si)	1·1–6·0	3·5
Germanium (Ge)	2·0–12·0	4·0

constant). Figure 4 shows how the depth of penetration varies with wavelength at three different angles of incidence for a germanium crystal. Since the depth of penetration is also a function of the difference in refractive index between the sample and the crystal, the slopes of curves such as those in Fig. 4 are different for other crystal materials.

Finally, the absorption coefficient and concentration of the absorbent affect the energy loss. For a given cell design and crystal in a given spectrometer, the attenuation of the beam, usually expressed as an absorbance, is directly related to absorbent concentration.

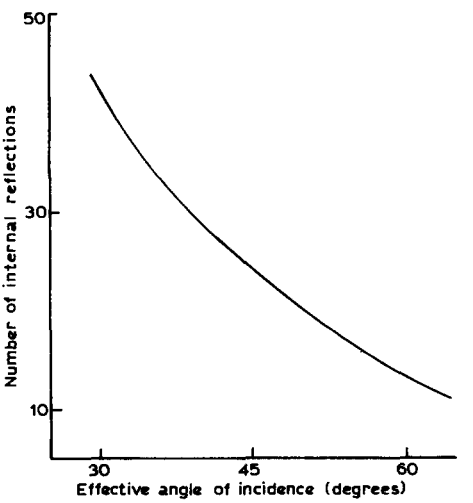

Fig. 3. The effect of angle of incidence at the crystal/sample interface on the total number of internal reflections (crystal dimensions: 50 mm × 2 mm thick).

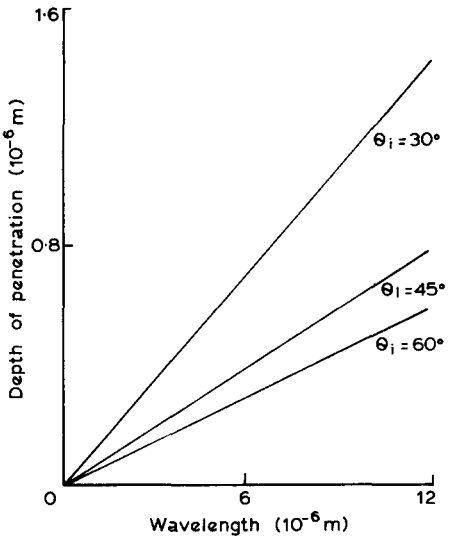

Fig. 4. The effect of wavelength on the depth of penetration of radiation into the sample for different angles of incidence (θ_i).

The MIR technique can be used for many applications which hitherto have involved the analysis of aqueous solutions for single or several components, a typical example being the analysis of milk and dairy products for protein, fat, carbohydrate, non-protein nitrogen and total solids.

EXPERIMENTAL

Materials

Lactose (analytical grade, Hopkin and Williams) was used as a standard. All other materials were plant derived, for which analytical data was obtained using the traditional analytical techniques (Lane & Eynon, 1923; Lillevik, 1970; Hubbard *et al.*, 1977). Where necessary these materials were reconstituted in distilled water by adding, in small amounts with gentle stirring, the required weight of material and diluting the solutions to the required volume. Compositions of the solutions are given in Table 2.

TABLE 2
Composition of Solutions Used to Calibrate the Analyser, Based on Analytical Data Provided for the Original Samples

Sample	*Composition of solution (%w/v)*				
	Total solids	*Fat*	*Total protein*	*Non-protein nitrogen*	*Lactose*
Lactose	10·00	0·00	0·00	0·00	10·00
35% WPC[a]	10·00	0·46	3·71	0·36	4·73
55% WPC	16·00	0·99	9·06	0·91	4·05
76% WPC	22·00	2·03	16·43	0·98	0·48
Permeate[b]	5·20	0·02	0·15	0·15	4·34
Skim milk powder	18·00	0·15	6·16	0·39	9·90
UHT milk	12·30	3·80	3·23	0·32	4·29
UHT half-cream	20·10	12·37	2·97	0·32	3·93

[a] Whey protein concentrate.
[b] Whey ultrafiltrate.

Equipment

Throughout this study a Miran-80 computing quantitative analyzer fitted with a horizontal MIR attachment containing a zinc selenide crystal was used. This instrument has now been superseded by the Miran-980 (Buck Scientific Ltd) which has, *inter alia*, an improved microprocessor package and the ability to provide infrared spectra of a component in solution by subtraction of the solvent spectrum (see Fig. 5).

Determination of wavelengths

Using the lactose solution (high in carbohydrate), 76% whey protein concentrate (WPC) solution (high in protein) and UHT half-cream (high in fat), in turn, the Miran-80 was used, in its scanning mode, to provide infrared spectra from which characteristic wavelengths for the major components were identified. When these spectra were compared with the spectrum for water, a number of wavelengths were identified which were not characteristic of any specific resonance but which appeared to increase with increasing total solids concentration. These wavelengths were investigated as potential frequencies for total solids determination. Figure 5 shows the resultant spectrum, after subtraction

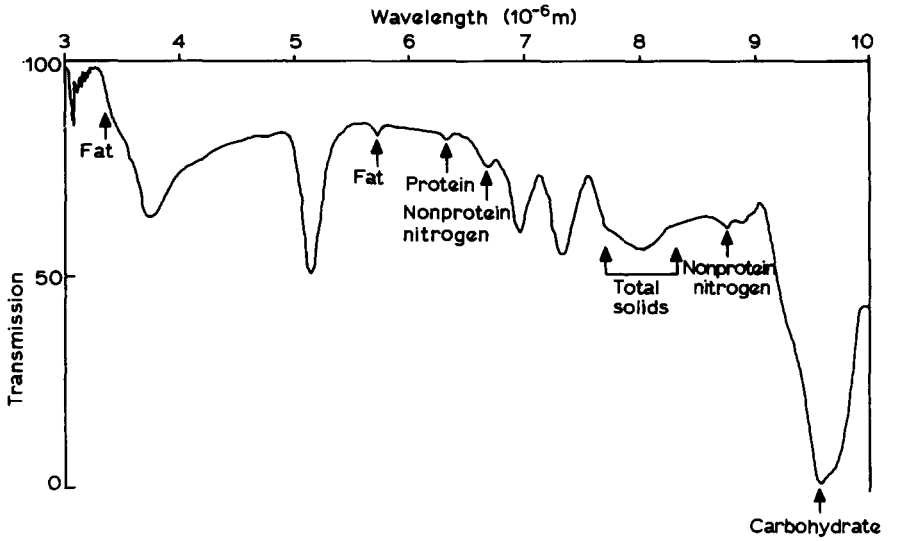

Fig. 5. Infrared spectrum of half-cream after automatic correction for water, using a Miran-980 analyzer. The component resonances are indicated.

of the spectrum of water, for half-cream. This corrected spectrum was obtained using a Miran-980 analyzer, fitted with the same horizontal MIR attachment.

Four-component analysis

The Miran-80 was programmed to analyse each sample at five wavelengths, namely a reference wavelength ($3{\cdot}97 \times 10^{-6}$ m, to compensate for the effects of changes in temperature, etc.) and the chosen wavelength for total solids ($8{\cdot}25 \times 10^{-6}$ m), fat ($5{\cdot}74 \times 10^{-6}$ m), total protein ($6{\cdot}35 \times 10^{-6}$ m) and carbohydrate ($9{\cdot}54 \times 10^{-6}$ m). The absorbances at each wavelength for each standard solution and the known concentration of each component in each standard solution were used to derive a calibration ('P') matrix, using a laboratory microcomputer. This microcomputer was also programmed to provide 'predicted' results for the same (standard) solutions, assuming the Miran-80 was programmed to use calibration curves with or without zero intercepts.

In normal use, the 'P' matrix has to be generated by microcomputer and manually entered into the Miran-80 to calibrate the instrument and, once this is done, the instrument requires no further programming, only

readjustment of the zero level. The in-built microprocessor in the Miran-80 will convert each absorbance to the corresponding concentration. The Miran-980 has the facility to generate the 'P' matrix without the need for a separate microcomputer.

Five-component analyses

The Miran-80 was reprogrammed to include a wavelength ($8{\cdot}80 \times 10^{-6}$ m) for non-protein nitrogen to give the initial five-component analysis. A further reprogramming, to give a modified five-component analysis, was undertaken using an alternative wavelength ($6{\cdot}68 \times 10^{-6}$ m) for non-protein nitrogen. The results for each system were again analysed by matrix manipulation to provide the predicted results for the solutions using zero and non-zero intercept calibration curves.

Interference by adsorption

A sample was analysed and allowed to remain in contact with the crystal for 2 h under a cover to prevent evaporation prior to being reanalysed. The MIR crystal was subsequently rinsed with distilled water and a water 'blank' reanalysed.

RESULTS AND DISCUSSION

Wavelengths for quantitative analysis

A number of possible characteristic analytical wavelengths were identified (Table 3) from the infrared spectra and, from the absorbances at each of these wavelengths for all eight samples identified in Table 2, the graphs of absorbance against concentration were plotted. From these graphs the optimum wavelengths were selected as wavelengths of choice, using the criterion of greatest response (i.e. steepest gradient) coupled with maximum linearity. The instrument resolves absorbance at any one wavelength from contributions due to absorbances at other wavelengths using a process of matrix inversion.

The wavelengths chosen for protein ($6{\cdot}35 \times 10^{-6}$ m) and carbohydrate ($9{\cdot}45 \times 10^{-6}$ m) showed good linearity whilst only the $5{\cdot}74 \times 10^{-6}$ m wavelength gave a linear response for fat. The two wavelengths chosen

TABLE 3
Potential Analytical Wavelengths Selected

Wavelength (10^{-6} *m*)	*Designation*
3·35	Fat
3·97	Reference
5·74	Fat
6·35	Total protein
6·68	Non-protein nitrogen
7·65	Total solids
8·25	Total solids
8·35	Total solids
8·80	Non-protein nitrogen
9·54	Carbohydrate

for non-protein nitrogen both gave linear responses but the longer wavelength ($8{\cdot}80 \times 10^{-6}$ m) was preferred, as the shorter ($6{\cdot}68 \times 10^{-6}$ m) showed some interference with the adjacent solvent (water) resonance. A range of wavelengths ($7{\cdot}47 \times 10^{-6}$ to $8{\cdot}35 \times 10^{-6}$ m) appeared to be related to non-specific total solids concentration and $8{\cdot}25 \times 10^{-6}$ m gave the greatest response to carbohydrate. From these wavelengths, one four-component and two five-component systems were devised and tested.

Four-component analysis system

Using the laboratory microcomputer to analyse the absorbance data produced by the instrument, the concentrations of fat, protein, carbohydrate and total solids were determined using zero and non-zero intercepts for the calibration curves (Table 4). Good agreement with the analytical values (Table 2) was obtained except for the determination of fat which only agreed at concentrations over 0·4%. The best agreement for all components was obtained by using non-zero intercepts for the calibration curves. Comparison of fat determination with the solvent extraction method (Hubbard *et al.*, 1977) can be expected to reflect the variable results known to be obtained with solvent extraction methods (Usher *et al.*, 1973) due to ionic interactions preventing the complete extraction of fat.

The Miran-80 was subsequently programmed, via the keyboard, with

TABLE 4
Computer Predicted Results for the Calibration Samples Using Four-Component Analysis System

Sample	*Predicted composition (%w/v)*			
	Total solids	*Fat*	*Total protein*	*Lactose*
(*a*) *Zero intercept*				
Lactose	9·64	0·56	0·00	8·93
35% WPC	10·59	0·80	4·26	4·48
55% WPC	15·28	0·41	8·82	4·13
76% WPC	22·08	2·45	16·40	0·08
Permeate	5·10	0·35	0·34	4·13
Skim milk powder	18·04	0·00	6·14	10·60
UHT milk	13·17	3·92	3·25	5·08
UHT half-cream	19·80	12·19	2·94	3·84
(*b*) *Non-zero intercept*				
Lactose	9·63	0·52	0·00	8·96
35% WPC	10·52	0·56	4·15	4·67
55% WPC	15·35	0·66	8·94	3·92
76% WPC	22·03	2·27	16·32	0·22
Permeate	4·90	0·00	0·03	4·69
Skim milk powder	18·12	0·00	6·25	10·39
UHT milk	13·19	3·99	3·28	5·03
UHT half-cream	19·82	12·23	2·96	3·80

the corresponding concentration ('P') matrix for non-zero intercepts. The results of five consecutive analyses of each standard solution were obtained and the mean and variance for each component are recorded in Table 5. The results show good agreement with the results obtained by the laboratory microcomputer ('predicted' results, Table 4) and reasonable agreement with the analytical values (Table 2) except for very low concentration levels. In the industrial application of the Miran-80 the level of agreement could be substantially improved by using a more restrictive definition of sample such that, for example, only milk, cream or whey protein concentrates are considered (Biggs, 1979) and many different samples (at least ten) of the plant-derived materials are used for calibration.

When extraneous material, such as sodium chloride for which the analyser is not programmed, is added to the sample, the calibration values

TABLE 5
Analytical Results Obtained Using the Four-component Analysis System

Sample	*Measured composition* ($x \pm$ %w/v)			
	Total solids	*Fat*	*Total protein*	*Lactose*
(*a*) *Calibration solutions*				
Lactose	9·50 ± 0·06	0·08 ± 0·04	0·24 ± 0·05	8·94 ± 0·05
35% WPC	8·96 ± 0·05	0·04 ± 0·04	3·36 ± 0·05	4·60 ± 0·00
55% WPC	14·26 ± 0·14	0·52 ± 0·04	8·18 ± 0·12	3·80 ± 0·13
76% WPC	19·98 ± 0·15	1·36 ± 0·10	15·30 ± 0·09	0·30 ± 0·00
Permeate	4·12 ± 0·04	0·00 ± 0·00	0·00 ± 0·00	4·50 ± 0·00
Skim milk powder	19·36 ± 0·05	1·34 ± 0·05	6·50 ± 0·00	10·14 ± 0·05
UHT milk	13·72 ± 0·04	4·62 ± 0·10	3·04 ± 0·10	5·16 ± 0·05
UHT half-cream	17·78 ± 0·07	11·02 ± 0·07	2·18 ± 0·10	3·74 ± 0·05
(*b*) *Other solutions* (*calculated value in parentheses below each value*)				
35% WPC[a]	9·20 ± 0·06	0·04 ± 0·05	3·46 ± 0·05	4·70 ± 0·00
(different sample)	(10·0)	(0·5)	(3·6)	(4·7)
35% WPC[a]	13·58 ± 0·07	0·66 ± 0·05	3·48 ± 0·07	8·44 ± 0·05
+ 5% lactose	(15·0)	(0·5)	(3·6)	(9·7)
35% WPC[a]	8·82 ± 0·07	0·40 ± 0·00	2·56 ± 0·05	4·60 ± 0·00
+ 5% sodium chloride	(15·0)	(0·5)	(3·6)	(4·7)

[a] This sample was prepared from material different from the calibration standard; the calculated values are derived from analytical results provided for this particular sample.

do not hold and, for example, total solids are not additive. When samples contain increased quantities of one component (as in the case of added carbohydrates) both that variable and the total solids values increase (see Table 5), indicating that the system can only operate within one application without the need for recalibration.

Five-component analysis systems

The initial five-component system used the same wavelength as in the four-component system with the addition of the wavelength $8{\cdot}80 \times 10^{-6}$ m for the determination of non-protein nitrogen. The computer predicted results (Table 6) show that a slight loss of accuracy has been introduced into the predicted results for total solids, total protein and carbohydrate whereas the previous results for fat content show improved correlation with the analytical values of the calibration solutions.

A modified version of the five-component system, using the alternative wavelength for non-protein nitrogen ($6{\cdot}35 \times 10^{-6}$ m), gave predicted

TABLE 6
Computer Predicted Results for the Calibration Samples Using the Five-component Analysis System (Initial Method)

Sample	*Predicted composition (% w/v)*				
	Total solids	*Fat*	*Total protein*	*Non-protein nitrogen*	*Lactose*
(*a*) *Zero intercept*					
Lactose	8·65	0·00	0·00	0·00	8·93
35% WPC	10·03	0·48	3·79	0·33	4·65
55% WPC	15·65	0·93	8·92	0·90	3·78
76% WPC	21·65	2·03	16·31	0·96	0·22
Permeate	5·22	0·17	0·17	0·10	4·44
Skim milk powder	19·13	0·12	6·49	0·46	10·80
UHT milk	12·48	3·97	3·36	0·26	4·42
UHT half-cream	20·10	12·31	2·94	0·34	3·93
(*b*) *Non-zero intercept*					
Lactose	8·87	0·00	0·00	0·00	9·11
35% WPC	10·19	0·45	3·82	0·35	4·78
55% WPC	15·61	0·94	8·91	0·90	3·75
76% WPC	21·78	2·00	16·34	0·97	0·32
Permeate	5·63	0·09	0·27	0·16	4·76
Skim milk powder	18·59	0·22	6·36	0·39	10·38
UHT milk	12·98	3·87	3·48	0·32	4·81
UHT half-cream	19·91	12·34	2·90	0·31	3·78

results (see Table 7) which showed very good correlation with the analytical values for total solids, total protein and carbohydrate whilst the fat and non-protein nitrogen values were significantly worse than were obtained with the initial five-component system.

Comparison of results from the four- and five-component systems indicates how the analysis is affected by interference between absorbances at the different wavelengths. Thus, the wavelengths have to be selected on a basis of compromise between the overall accuracy of the system and determination of the maximum number of components. A drawback to using the modified five-component system with the Miran-80 is that the microprocessor in the instrument is unable to convert the absorbance values into concentration values because it cannot cope with the severe non-diagonal properties of the concentration matrix. Hence, for the

TABLE 7
Computer Predicted Results for the Calibration Samples Using the Five-component Analysis System (Modified Method)

Sample	*Predicted composition (%w/v)*				
	Total solids	*Fat*	*Total protein*	*Non-protein nitrogen*	*Lactose*
(*a*) *Zero intercept*					
Lactose	10·30	0·48	0·12	0·06	9·63
35% WPC	10·17	0·85	4·03	0·40	4·02
55% WPC	15·50	0·38	8·95	0·80	4·37
76% WPC	22·22	2·43	16·48	1·02	0·22
Permeate	4·81	0·39	0·18	0·08	3·81
Skim milk powder	17·83	0·00	6·02	0·34	10·37
UHT milk	12·84	3·96	3·07	0·41	4·73
UHT half-cream	19·87	12·18	2·98	0·27	3·91
(*b*) *Non-zero intercept*					
Lactose	10·31	0·24	0·06	0·06	9·90
35% WPC	10·18	0·70	3·99	0·40	4·19
55% WPC	15·49	0·60	9·00	0·81	4·12
76% WPC	22·23	2·19	16·42	1·02	0·50
Permeate	4·84	0·00	0·00	0·07	4·60
Skim milk powder	17·81	0·00	6·11	0·35	9·97
UHT milk	12·83	4·13	3·11	0·42	4·53
UHT half-cream	19·87	12·21	2·99	0·27	3·87

Miran-80, the choice of system is between the accuracy of the four-component system and the extra data available from the initial five-component system.

The more recent Miran-980 analyser, introduced by the manufacturers after completion of this study, has a greater mathematical capability which allows the modified five-component system to be adopted. The Miran-980 instrument was introduced purely as a superior laboratory-based multi-functional analyser which is able to perform many other functions such as recording the infrared spectra of solutes after subtraction of the solvent spectrum and storage of spectra for future use. The Miran-980 is therefore not the type of instrument which would normally be considered for a plant situated analyser, but the method of wavelength selection, sample introduction and calibration are exactly the

TABLE 8
The Effect of Long-term Contact of Samples with MIR Crystal

Wavelength	*Absorbance*	
	Initial	*After 2 h*
3·35	0·068 5	0·072 6
3·97	0·016 2	0·016 4
5·74	0·201 1	0·211 3
6·35	0·270 2	0·282 6
8·25	0·012 9	0·019 1
9·54	0·080 0	0·092 1

same as for the Miran-80, thereby providing an alternative analyser for a laboratory-based system.

Interferences by adsorption

Because the MIR system is based on determination within only a small thickness of liquid adjacent to the MIR crystal, it is essential that the crystal has no effect on the sample, such as preferential adsorption of components. A sample left under cover on the MIR crystal for 2 h showed changes in absorbance (Table 8) equivalent to changes in concentration of 0·1 %w/v. The change in absorbances obtained for the subsequent analysis of a water 'blank' gave further indication that the level of material which is absorbed by the MIR crystal is not significant and represents concentration value changes less than those reported for duplicate analyses using chemical methods (i.e. less than $\pm 0{\cdot}1\,\%$).

CONCLUSIONS

A number of dedicated infrared analysers have been introduced onto the market, including Multispec (Shield Instruments, York, Great Britain) Milko-Scan and Milko-Tester (both A/S N. Foss Electric, Hillerod, Denmark), all of which use very similar techniques to determine fat, protein, carbohydrate and solids (Multispec and Milko-Scan) or fat (Milko-Tester) in milk products. The basic technique has been evaluated and the agreement with chemical methods found to be within normally

accepted limits, particularly when calibrations are restricted to the specific type of sample to be analysed (Biggs, 1979).

The major difference between the Miran-80 and the Multispec and Milk-Scan analysers is that the calibration of the Miran-80 is not restricted to preset wavelengths chosen by the manufacturers or to user-variable wavelengths achieved by varying a limited range of expensive filters. The Miran-80 can be programmed by the user to the exact wavelengths required to suit the specific application due to the use of a continuously variable filter wheel system. This has the added advantage that the analyser does not become obsolete should the user cease to analyse milk products; the Miran-80 can readily be reprogrammed to monitor such materials as ethanol, carbon dioxide or many other specific components which have characteristic infrared wavelengths.

The Miran-80 provides a simple method for the simultaneous determination of the milk and dairy product components: fat, protein, non-protein nitrogen, carbohydrate and totals solids. The method, which requires no arithmetic calculation, can be operated by non-laboratory staff with a minimum of training without sample preparation to provide a full analysis in under 2 min. Such a system provides a relatively cheap and simple method for the routine analysis and control of good processes.

ACKNOWLEDGEMENT

The authors are grateful to Dr S. H. Bruce of Foxboro Analytical, Milton Keynes, Great Britain, for technical assistance and co-operation.

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