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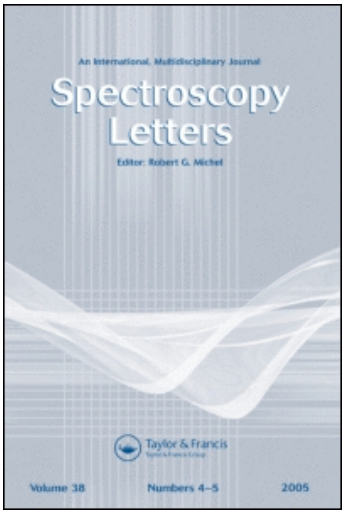


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# Mid-Infrared Transmission Spectroscopy of Sugar Solutions: Instrumentation and Analysis

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# MID-INFRARED TRANSMISSION SPECTROSCOPY OF SUGAR SOLUTIONS: INSTRUMENTATION AND ANALYSIS

Keywords: IR spectroscopy, carbohydrate, aqueous, transmission

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## ABSTRACT

Transmission studies on a specially designed scanning spectroprocessor for aqueous systems are reported for the first time. The nature of the absorption bands and the possibility of a universal isobestic point are discussed for a series of sugar solutions.

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## INTRODUCTION

Infrared spectroscopy is a valuable technique for the identification and characterization of substances found in nature and has been used as a tool not only for the study of molecular structures but also as a method for quantitative component analysis[1-3] . The practical difficulties of examining the infrared spectra of aqueous solutions have however deterred spectroscopists from using water as a solvent or studying biological systems. Water shows very strong absorption bands throughout the IR region so that in spectra of aqueous solutions, absorption bands due to the solute are usually obscured unless special instrumentation is employed. In one of a series of publications, Goulden[4] presented the experimental difficulties and techniques involved in the study of infrared spectra of aqueous solutions. His work led to the development of infrared analysis for fat, protein and lactose determination in milk products.

In the case of milk, the situation is relatively uncomplicated in regards to carbohydrates since the only sugar of consequence is lactose. However, in other systems, a variety of simple and compound sugars plus polysaccharides are often present. Analysis, differentiation, and quantitation of the simple sugars by the infrared method would be of major interest for analytical, quality control and formulation purposes.

Among the studies made on the infrared spectra of various carbohydrates, those of Kuhn[5], Whistler and House[6], Barker et

al.[7] and Parker[8] are the most noteworthy. However, because of the differences in the preparation of the samples, it is difficult to compare the reports and spectra which have appeared in the literature. The simple fact that there is a shift in frequency and a tendency for the absorption bands to broaden when a sample passes from the crystalline to the amorphous state makes it difficult to ascertain if certain of the absorptions reported were attributable to the sugar or to the physical state of the sample. Since a unique instrument, the Spectroprocessor IV, specially designed for aqueous systems was available, a study was undertaken with the objective of obtaining spectra and possibly identifying an isobestic point, i.e., a wavelength at which the simultaneous analysis of a wide range of sugars in solution would be possible.

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## Materials and Methods

The following sugars were obtained for analysis: D-glucose (Certified A.C.S. anhydrous, sp. rot. 25 $^{o}$ = +53$^{o}$, Fisher Scientific D-16), alpha-lactose (Certified A.C.S., Fisher Scientific L-5), D-galactose (Purified crystalline anhydrous, Sigma G-0625), D-mannose (Mixed anomers anhydrous, beta-anomer 21%, alpha-anomer 78%, Sigma M-4625), D-fructose (Reagent grade anhydrous, sp. rot. 20$^{o}$ = -92.4 + or - 0.5$^{o}$, Fisher Scientific L-95), sucrose (Certified A.S.C., sp. rot. 25$^{o}$ = 66.4, Fisher Scientific S-5), and maltose (sp. rot. 20$^{o}$ = +127 to 132$^{o}$, BDH 29131). In addition, soluble starch (Certified A.C.S., pH 6,

Fisher Scientific S-516) was scanned for comparative purposes. Five percent solutions (w/v) of each sample were prepared and scanned on the Spectroprocessor IV. The Spectroprocessor IV was custom engineered for our research purposes by Mr. J. Shields, Shields Instruments Ltd, York, England. The instrument is based on the use of a Grubb Parsons Spectromaster chassis [9] which was rebuilt optically and electronically with the following modifications:

a) gold plating replaced the aluminum coated mirrors to enhance reflection;

b) a 6:1 ellipsoid mirror was use in conjunction with a high TGS pyroelectric detector with its own built in condensing lens;

c) the original gratings were replaced by new ones, the first having 984 lines/cm, blazed at 11 um to cover the 5-25 um range in the first order, the second having 2,953 lines/cm, blazed at 3.5 um to cover the 2-5 um range in the first order and the 0.5 to 2 um range in the second order. The double monochromator (Czerny-Turner grating, prism and Littrow mirror) system was retained because of its efficiency in reducing stray light;

d) the original comb and servo drive were replaced by a substantially larger comb and a highly responsive servo motor which minimize dead space at the null balance point.

e) the original amplifier was also discarded for a high performance low noise solid state amplifier more sensitive to minute changes in signal. These modifications are especially useful for the low energy situations arising in aqueous system IR work.

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f) the original slit and grating drive motors and cam systems were replaced by digitally controlled stepping motors, the grating being driven by a Berger-Lahr five phase stepping motor (1,000 steps/revolution) on a 1:1 basis with the grating drum, i.e., 1,000 steps per grating micrometer revolution; the slits being driven by an Astrosyn four phase stepping motor (400 steps/revolution) on a 2:3 basis with the slit micrometer, i.e., 600 steps per slit micrometer revolution.

g) the instrument cell holders were modified to house short path length flow through cells (16 and 36 um). Two sets of windows were provided, barium fluoride and strontium fluoride, these materials being water-insoluble and having transmission cut-offs at approximately 12.5 and 10 um respectively. The sample cell holder was plumbed with 0.1 cm i.d. stainless steel tubing to allow the fluid sample to be pumped through the cell. A homogenizer pump and block was set up beside the instrument for homogenizing and pumping the sample through the cell if desired. A portion of the cover of the instrument was cut out and replaced with a removable glass plate to allow ready access to the cells.

h) the instrument was hermetically sealed and provided with two ports. A peristaltic pump with tubing was attached to these ports, passing through two four liter containers of dried molecular sieve. This addition served to dry the instrument to prevent loss of energy due to moisture. The base plate of the instrument was provided with solid state heaters to provide an

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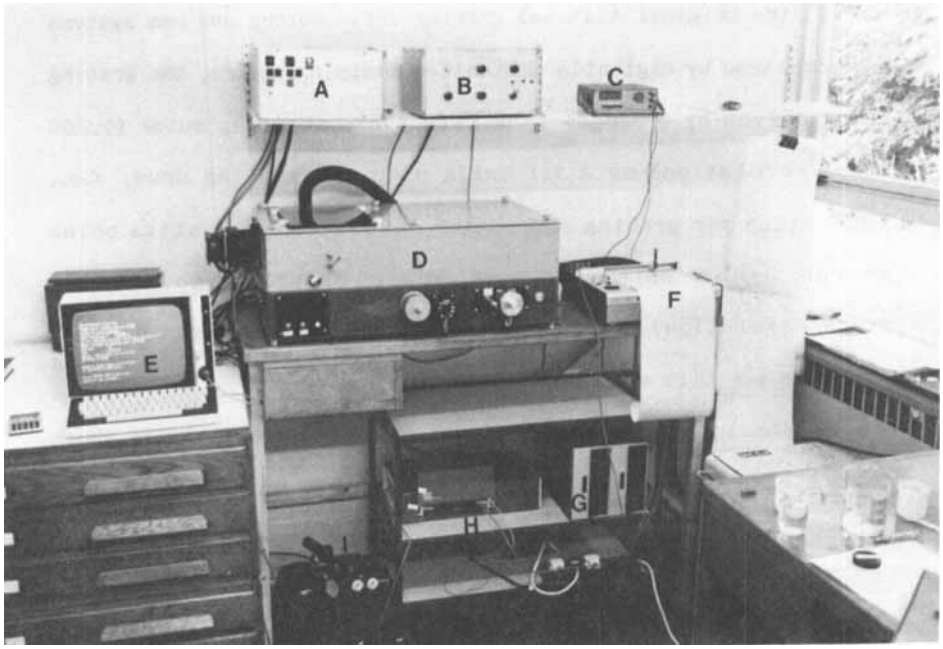


FIG.1 The Spectroprocessor IV.

(A) Stepping motor control
(B) Solid state amplifier
(C) Digital voltmeter
(D) Spectrophotometer
(E) CRT display unit
(F) Strip chart recorder replaced by x,y, plotter
(G) Disk drives
(H) Zilog microprocessor
(I) Pump for dessicating instrument
(K) Hard copy terminal for data outpout (not shown)

internal temperature of 37°C monitored by a direct readout thermometer housed near the cells.

Basically the instrument (Fig.1) addresses the limitations found in many standard IR spectrophotometers designed for use with IR transparent solvents, i.e., high energy source, removal of water vapor, temperature stability and flow through cells.

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Instrument logic and data processing

The Spectroprocessor IV is controlled by a Zilog MC Z80 microprocessor and operated from a CRT terminal. The microprocessor has a total capacity of 64K memory and is equipped with a dual disk drive accessory. One disk drive is used to house the instrument's operational control programs and calibration files while the second is used for data storage. The control programs allow the manipulation of the stepping motors driving the slit and gratings and contain the command language (RIO operating system) which enables one to set up the instrument in any manner desired. The data stored on floppy disk consist in the amplified analog signal obtained from the movement of the instrument's comb in the form of digitized voltages ranging from 0-10 volts, the equivalent of transmission reading. Each disk can store 1:1 megabytes which is about 500,000 digits. These digits can be printed on an auxiliary printer or plotted to produce a spectrum.

One of the major features of this instrument's program capabilities is that a wide range of slit programs can be developed. Most IR instruments have a mechanical slit program which attempts to maintain constant energy as a spectral range is scanned. The use of IR transmitting solvents such as carbon tetrachloride and carbon disulphide require simple slit programs. Water, however, which absorbs throughout the IR region causes varying energy problems and obtaining constant energy is a major difficulty. This has been obviated to a large extent with this instrument by the generation of calibration programs. A

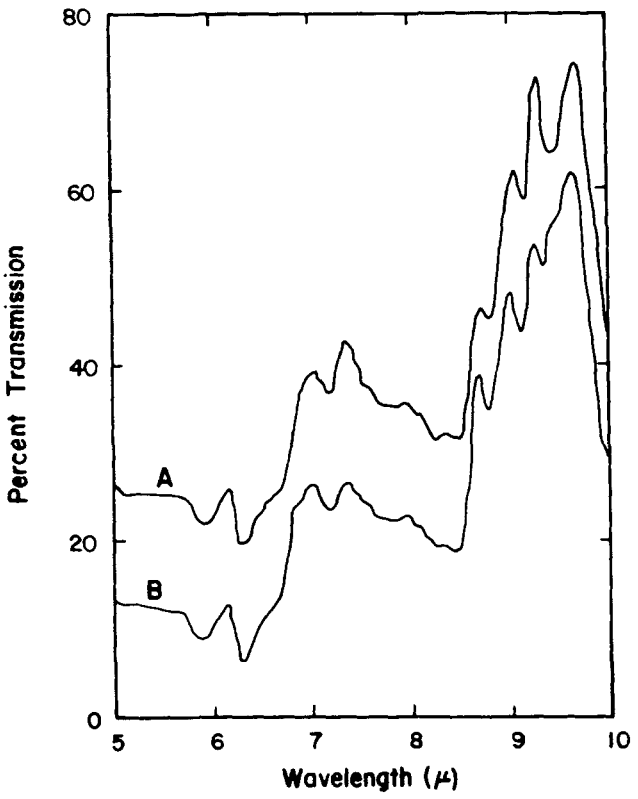


FIG. 2. Scan of glucose showing the mutarotation effect.

(A) Immediately after dissolution in water, the doublet peaks representing alpha and beta anomers.
(B) After 24 hours, where the alpha anomer is predominant.

calibration program allows one to obtain a constant energy level, e.g., 50%, by slits opening and closing automatically as the grating moves to each new position. A calibration file is generated by scanning the solvent, in this case, water, in the single beam mode and storing the slit settings on disk for a desired energy level. The calibration program is then run in

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tandem with a scan of the solution under study. This practice assures constant energy from scan to scan. One is capable of sampling data, wavelength, slit, etc. during a scanning operation by reading the video display or alternatively one can go to a specified wavelength and sample the signal on a continuing basis.

## Results and Discussion

One of the first problems encountered in this work was that of the well known mutaroration effect as illustrated in Figure 2. The difference in spectra in the 9 to 10 um range is due to mutarotation which occurs when a reducing sugar is dissolved in water. D- glucose mutarotates in water to give a mixture of alpha and beta glucose which differ in the fact that the alpha-D-anomeric hydrogen occupies the equatorial position and the hydroxyl occupies the axial position whereas in the beta form, the anomeric hydrogen occupies the axial position and the hydroxyl, the equatorial position. The beta isomer absorbs at a lower wavelength than the corresponding anomeric alpha form since the hydroxyl in the equatorial position interacts with the oxygen on C-5 to increase the frequency. These doublet peaks between 9.4 and 9.8 um were also observed by Kuhn[5], Whistler and House[6] and Parker[8].

This problem was not apparent in the remaining reducing sugars and was considered to be due to either more rapid equilibrium or to an initial equilibrated situation. Examination

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of the infrared spectra of the simple sugars showed that all sugars have a band at about 3.1 um due to the OH stretching frequency of the hydrogen bonded hydroxyl groups while in the 8 to 10 um, there were several closely spaced bands due to OH bending or deformation and C-O and C-OH stretching vibrations. These bands overlap to a degree and there are no highly individual bands for the specific group frequencies. The 9.61 um wavelength used for lactose determination in milk is characteristic of the C-OH stretching frequency in straight chain primary alcohols. In identifying the C-OH stretching frequency for other sugars (Fig. 3-5), one notes that the spectra are quite different either in intensity or frequency. These differences could be due to overlapping and summation of closely situated bands of the two anomers, to the extent of hydrogen bonding or to the number of C-O groups in the vicinity. Although all the hexose sugars have the same carbon to hydrogen to oxygen ratio, differences in the arrangement of these atoms in the molecule and differences in conformation introduce significant differences in their infrared spectra. Because of this, quantitative analysis of aqueous solutions of mixtures of different sugars is difficult and complex.

Three particular regions were observed where some of the 5% solutions had similar absorbancies. These sugars and their absorbances at 8.95, 9.0 and 9.61 um are presented in Table 1. Also tabulated are the estimated concentrations one would obtain using the lactose absorption as a base reference.

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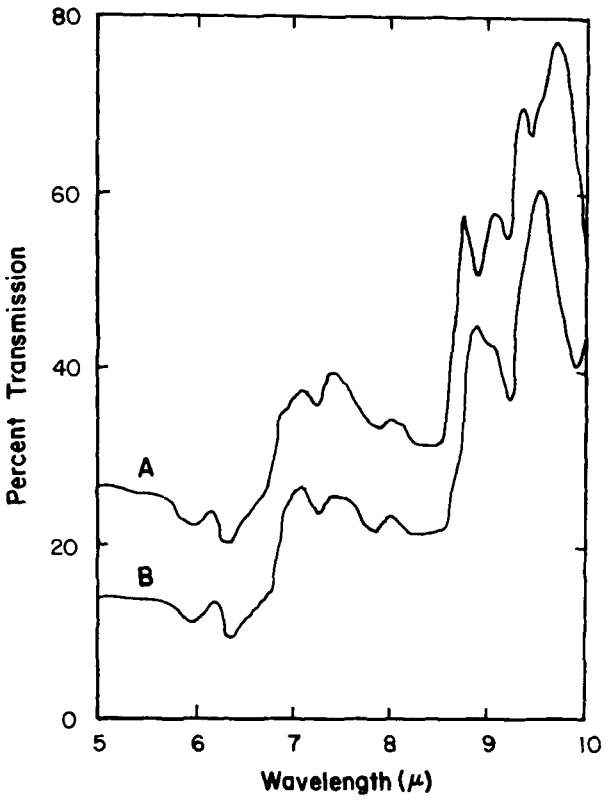


FIG. 3. Typical scan of maltose (A) and sucrose (B).

It is apparent that none of the wavelengths truly serve as an isobestic point for all of the sugars in question. The 8.95 um wavelength would serve well for glucose and maltose or alternatively for lactose and mannose. The 9.0 um wavelength serves best overall, showing the least overall SD. Maltose and mannose could be analyzed with this wavelength. The 9.61 wavelength is the worst overall for general sugar analysis,

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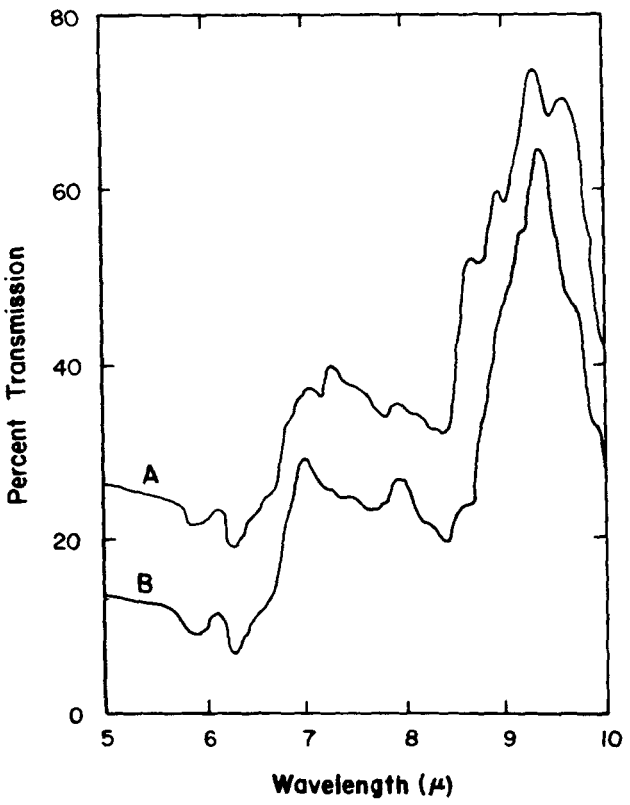


FIG. 4. Typical scan of lactose (A) and mannose (B).

lactose and glucose however, having a common absorptivity here. In assessing these three wavelengths, fructose is definitely a sugar which causes the greatest discrepancy. For general sugar analysis, the most common sugar combination found in foods is that of sucrose, glucose and fructose, the latter two often being added or present as hydrolysis products. None of the wavelengths chosen seem to be capable of handling this situation.

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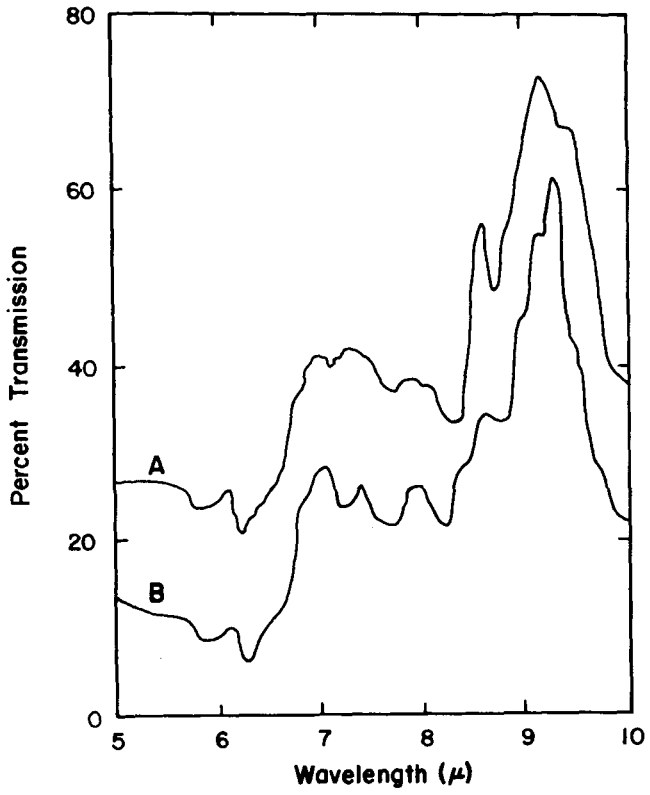


FIG. 5. Typical scan of galactose (A) and fructose (B).

## Conclusion

It is recognized that this study has basic limitations, i.e., it assumes the analysis is only being made on the pure sugars or potential mixtures thereof. However, based on this work, sufficient data were obtained to infer that the concept of obtaining a true isobestic point for all sugars is improbable. In the 8 to 10 um region, the wavelengths are not fundamentally

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TABLE 1

Absorbances of Standard 5% Sugar Solutions and Estimated % Concentration using Lactose as Reference

| Sugar | A(8.95) | Conc. | A(9.00) | Conc. | A(9.61) | Conc. |
|---|---|---|---|---|---|---|
| Lactose | 0.537 | 5.00 | 0.537 | 5.00 | 0.397 | 5.00 |
| Sucrose | 0.585 | 5.44 | 0.585 | 5.44 | 0.366 | 4.60 |
| Glucose | 0.568 | 5.28 | 0.508 | 4.72 | 0.397 | 5.00 |
| Maltose | 0.568 | 5.28 | 0.523 | 4.86 | 0.318 | 4.00 |
| Mannose | 0.552 | 5.13 | 0.523 | 4.86 | 0.494 | 6.22 |
| Fructose | 0.698 | 6.49 | 0.602 | 5.60 | 0.568 | 7.15 |
| Galactose | 0.795 | 7.40 | 0.552 | 5.13 | 0.420 | 5.28 |
| Mean | | 5.71 | | 5.08 | | 5.32 |
| SD | | 0.88 | | 0.32 | | 1.05 |

identifiable as are the 5.74 for carbonyl and the 6.46 for amide II absorptions when analyzing for fat and protein. Consideration should also be given to the fact that a potential isobestic point would most probably lie on the steep shoulder of the absorption peak for some sugars, leading to large errors if there were any shifts in frequency due to environmental conditions. e.g., changes in hydrogen bonding. This spectral region is also affected by many other compounds in unpredictable ways and in a complex food system, quantitation for carbohydrate would be lost.

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