Essential Absorption Data for In-vitro and In-vivo Near Infrared Spectrometric Biotic Fluid Assays

H.M. Heise and A. Bittner

Institut für Spektrochemie und Angewandte Spektroskopie,
Bunsen-Kirchhoff-Str. 11, D-44139 Dortmund, Germany

The performance of near infrared spectroscopic assays for the analysis of biotic samples within the frame of clinical chemistry has been the subject of several investigations. The determination of metabolites such as glucose can be carried out using multivariate calibration exploiting spectral data from different intervals. The reliability of such assays can be improved if special boundary conditions are fulfilled, as the existence of other components with similar spectra can lead to severe systematic errors for concentration prediction. Absorptivity data between 11000 and 3500 cm^{-1} is presented for the monosaccharides glucose, fructose and galactose, as well as for ethanol and hydroxyethylstarch used for hemodilution or as blood plasma expander. Pure compounds and aqueous solutions were studied. Since the hydrogen bonding network is considerably influenced by such solutes, also spectral information is provided on the variability of the spectra of aqueous samples due to temperature and electrolyte content of NaCl and KCl.

INTRODUCTION

Traditionally, infrared spectroscopy has been one of the most important physical methods in the chemical laboratory as it plays an important role in the elucidation of structures and the identification of organic compounds. Quantitative analysis has become another strength, in particular for process monitoring, which is dominated by near infrared spectrometry. Applications in clinical chemistry are growing because reagentless and fast spectrometric multicomponent assays are being developed. Promising results have been shown for near infrared spectrometry for several blood substrates (1-3). Furthermore, non-invasive diagnostic methods are desirable, as these would allow gentle and painless monitoring of important metabolites (4).

In this investigation important absorption data for the development of in-vitro and in-vivo near infrared spectrometric sensors are compiled for a broad spectral range from 11000 to 3500 cm^{-1}. Absorbance spectra are provided for different hexose sugars such as glucose, fructose and galactose, for ethanol and hydroxyethyl starch used for hemodilution or as blood plasma expander. For the sugars, spectra were recorded from crystalline powders, glassy samples prepared from sugar sirup and aqueous solutions. As the hydrogen bonding network of the water molecules within the aqueous solutions is affected by alcohol and carbohydrate solutes, other effects such as temperature variations and electrolyte concentrations (NaCl and KCl) on the spectrum of water are also investigated. A critical evaluation of the similarity between the spectra is given.

EXPERIMENTAL

Spectral measurements in the near infrared spectral range were carried out using a Bruker FT-spectrometer IFS 66 equipped with tungsten lamp, CaF_2 beamsplitter and InSb detector from Infrared Ass. (Suffolk, U.K.). For the aqueous solutions quartz transmission cells of 10 mm pathlength (short-wave near infrared) and of 0.5 mm (conventional near infrared) were used, which could be thermostatted to a temperature stability of ± 0.01 °C. Crystalline powders were measured using a diffuse reflectance accessory, which is attached via transfer optics to the spectrometer (5). The same InSb detector as for transmission measurements can be employed. Sample cups are adjustable in depth (5 mm was considered here). Glassy samples were prepared on diffusely reflecting gold-coated substrates of grade 1000 $inch^{-1}$ sandpaper from highly viscous sirup preparations and slow water evaporation by using a stream of warmed air. Such samples were also measured with the diffuse reflectance accessory. Usually, a spacer of 200 µm was used for pathlength setting, so that an average pathlength of 0.5 mm can be estimated for the transflection measurement, taking in account the solid angle seen by the detector and a Lambertian scattering characteristics for the substrates.

Glucose, fructose and galactose were for biochemical use and were supplied by Merck (Darmstadt, Germany). Ethanol, NaCl and KCl, each of grade *pro analysi*, were obtained from the same supplier. Hydroxyethyl starch (HES with average M_w 200000/ degree of substitution 0.5; 10 % in aqueous isotonic NaCl solution) was from Fresenius (Bad Homburg, Germany).

Ralf
Marbach

RESULTS AND DISCUSSION

The impetus for this investigation stems from our engagement in developing a non-invasive spectrometric blood glucose sensor for diabetic patients.This is desirable as monitoring is an important part of management of this disease. Frequent monitoring of blood glucose can help prevent complications from severe hypo- and hyperglycemia. For this reason glucose is the key compound in this investigation. Fructose is a ketohexose used as caloric sweetener which is more than two times sweeter than glucose and can be fitted into the diet of diabetic patients, because it does not raise the blood sugar as fast as glucose (the carbohydrate glycemic index for fructose is 20 compared to 100 for glucose). The third monosaccharide studied is galactose, an aldohexose which shows, like glucose, α- and β-anomeric forms. Galactose is, besides glucose, part of the lactose disaccharide found in milk and is bound to most glycoproteins. Hydroxyethyl starch (HES) solutions have been reported to have good characteristics for hemodilution to improve microcirculation of the blood or is used as blood substitute. Ethanol consumed as part of an alcoholic beverage can be found in similar concentrations in blood as glucose.

Quantitative absorption data on these compounds is still missing, although in some publications limited spectral

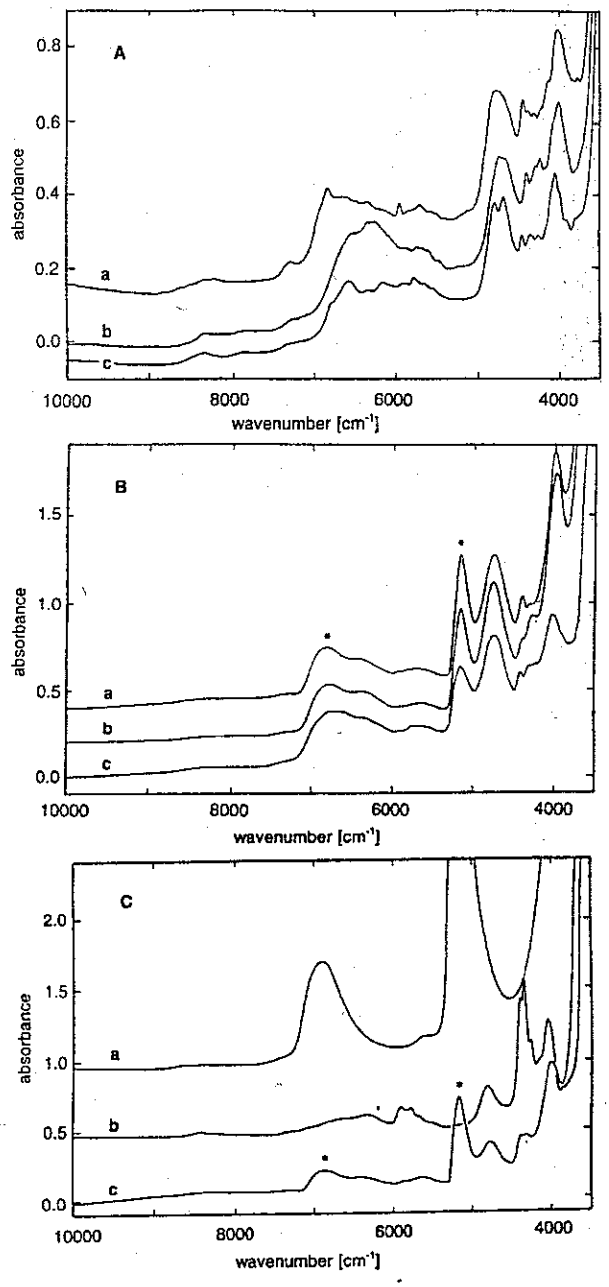

FIGURE 1. Comparison of pure component near infrared spectra: **A** Diffuse reflectance spectra of different crystalline monosaccharides: a) fructose, b) glucose and c) galactose; **B** spectra from glass-like sugar samples prepared from sirup preparations and measured in transflectance (same order as given in A); **C** absorbance spectrum of water (a) and ethanol (b), each recorded with 0.5 mm pathlength and at 25 °C, and transflectance spectrum of hydroxyethyl starch (c).

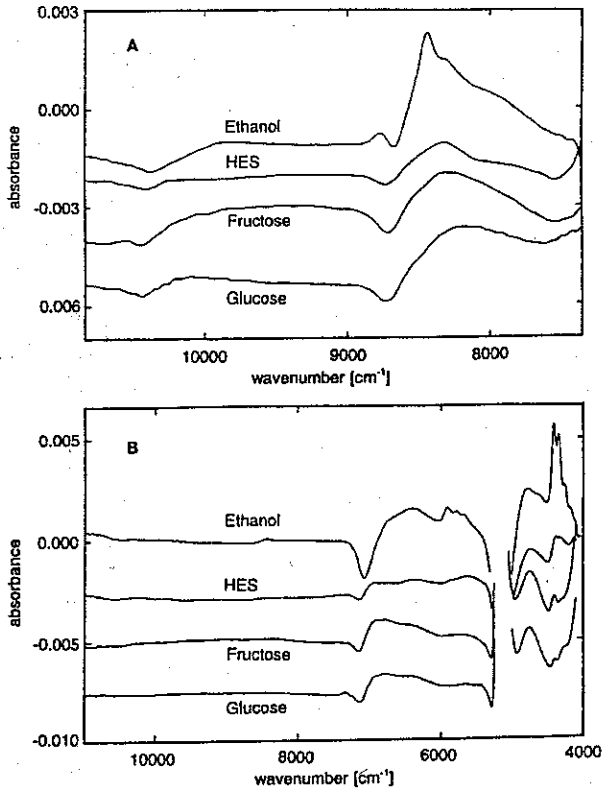

FIGURE 2. Difference spectra of some diluted aqueous solutions of compounds important for biotic assays with partial water absorbance compensation: **A** short-wave near infrared spectral range recorded from solutions of 0.5 % concentration each recorded at 30 °C with a 10 mm pathlength cell; **B** near infrared data from solutions of 1.5 % concentration recorded at 30 °C with a 0.5 mm pathlength cell.

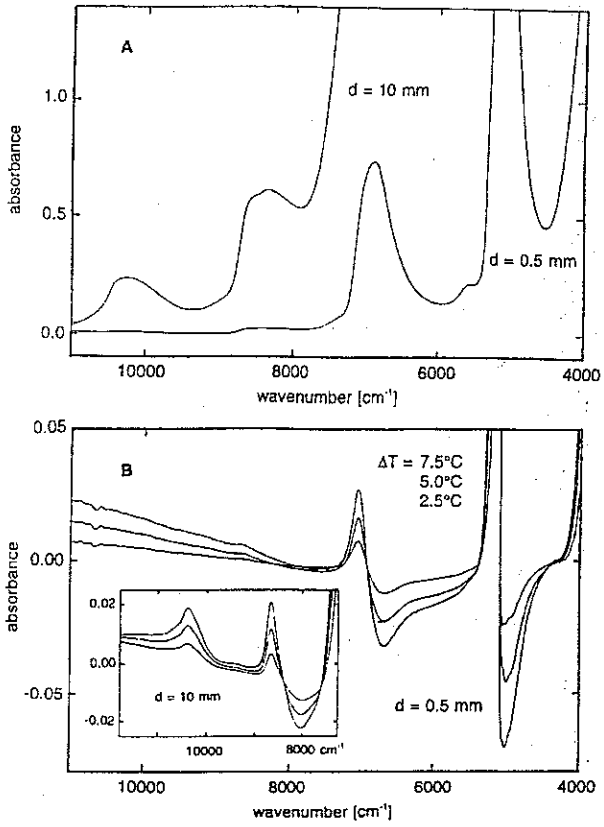

FIGURE 3. Near infrared absorbance spectra of water recorded at 25 °C (A), and difference spectra showing temperature dependencies (the reference water spectrum was recorded at 25 °C) (B).

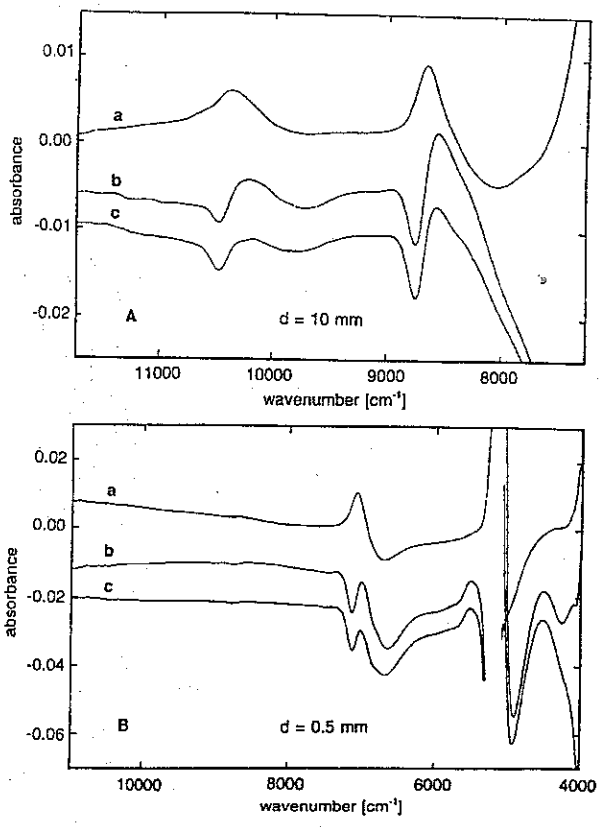

FIGURE 4. Near infrared difference spectra for water at 32.5 °C (a), for an aqueous solution of NaCl (b), and of KCl (c) (5 % each) versus a spectrum of water at 30 °C: A short-wave near infrared spectra recorded at 10 mm pathlength; B near infrared spectra recorded at 0.5 mm pathlength.

features are presented. This is in particular valid for aqueous solutions, for which the hydrogen bond network is changed, so that additional effects occur when scaled absorbance subtraction is applied for compensating the solvent absorption features.

As solids, the sugars show many sharp peaks in their spectra, see Figure 1A; however, with these compounds as solutions usually broad absorption bands appear. The influence of different aggregate states on the spectrum of glucose was recently studied by Reeves (6). Near infrared spectra of crystalline, molten, glass-like, freeze-dried, and saturated solution (water subtracted) of glucose were presented. The last three types of spectra show greatest similarity. Similar spectra have also been reported for the short-wave near infrared range (7).

We produced some glass-like modifications from sirup preparations which lead to solution equivalent spectra (compare Figures 1B, C and 2B). It is difficult to remove the remaining water without starting crystallization (residual water bands are marked by an asterisk). Water adsorbed to starch could, for example, only be removed after vacuum drying at 60 °C (8). For completeness Fig. 1C shows the spectra of water and ethanol recorded in a transmission cell, as well as the spectrum of HES measured as dried film. The comparison of the latter two spectra provide some clues for the assignment of vibrational bands.

There have been some efforts to quantify changes in the near infrared spectra of such compounds caused by the influence of water, pH, ionic strength and differences in the physical state using regression statistics, but these were limited to the long-wavelength interval between 4900 and 4100 cm^{-1}. Similarly, larger intervals could be tested, but especially in the latter region some fine structure is evident which can be used for compound discrimination.

The situation is similar with spectra from diluted solutions, for which some water compensation is applied. However, additional effects from changes in the hydrogen bond network compared to pure water can be seen from the first derivative features. Excluding the interval between 4500 and 4000 cm^{-1}, the spectral features for the monosaccharides and HES do not show significant differences. Using a multivariate approach, calibration models for ethanol will be less effected by spectral collinearity, although some interferences are still to be expected to the other compounds shown.

As we were interested in manifesting the O-H equilibrium structure perturbations, water spectra under different conditions were recorded. In Figure 3 the absorbance spectra of water at 25 °C for different pathlengths are shown. The effect from temperature changes is best illustrated by difference spectroscopy. One can see that the effects are linear with temperature change, so that temperature prediction can be based on such data; literature references on this subject can be found in (3).

The same can be stated for the effect that electrolytes have on the hydrogen bonding structure; for this reason only the spectrum of one NaCl and KCl solution is displayed in Figure 4. Spectral gaps above 5000 cm^{-1} are due to the high water absorbances seen even with a cell of 0.5 mm pathlength, so that the noise level becomes unacceptable. A much greater complexity of electrolyte samples has been recently studied by Brown and coworkers (9) who also give a good coverage of the literature available in this field. However, sodium and potassium ions are interesting from a physiological point of view because of some concentration variability.

Another parameter with influence on the spectrum is the pH value of water, which has recently been investigated by Molt and Cho (10). They followed a titration of an HCl-solution with NaOH. The resulting difference spectra show the same features as provided by Figure 4B (see trace b for features obtained for the NaCl-solution).

The expected large temperature influence on substrate concentration prediction has already been proved previously by us using partial least-squares calibration models from short-wave near infrared absorbance or logarithmized single beam spectra of blood plasma (11). When spectral differences against a water background spectrum recorded subsequently, the temperature impact on prediction could be eliminated. Improved difference spectra of biotic samples might be produced employing reference spectra of water with adjusted ionic strength.

CONCLUSIONS

To obtain reliable quantitative information on various analytes in tissue or blood, spectrometric methods are necessary which measure a broad spectral range allowing multivariate calibration modeling. Furthermore, certain behavior, such as avoiding fructose or alcohol, can assist in improving the reliability of glucose concentration prediction within diabetic patient self-monitoring. Restrictions for glucose have to be observed within clinical monitoring when hemodilution or the application of blood plasma expander with polysaccharide compounds has been considered for the patient. The same statements given for the in-vitro analysis of blood samples are valid for future non-invasive spectrometric in-vivo sensing systems.

ACKNOWLEDGEMENTS

Financial support by the Ministerium für Wissenschaft und Forschung des Landes Nordrhein-Westfalen and the Bundesministerium für Bildung, Wissenschaft, Forschung und Technologie is gratefully acknowledged. Further funds were granted by the Deutsche Forschungsgemeinschaft.

REFERENCES

1. Arnold, M.A., New developments and clinical impact of noninvasive monitoring' in: Handbook of Clinical Automation, Robotics, and Optimization, Kost, G.J., and Welsh, J., eds., New York: John Wiley & Sons, 1996, pp. 631-647
2. Heise, H.M., Mikrochim. Acta [Suppl.] **14**, 67-77 (1997)
3. Heise, H.M., Near-Infrared Spectrometry for in vivo Glucose Sensing, in: Biosensors in the Body: Continuous in vivo Monitoring, Fraser, D.M., ed., Chichester: John Wiley & Sons, 1997, ch. 3, pp. 79-116
4. Heise, H.M., Proc. IEEE Engineer. Med. & Biol., 18th Ann. Int. Conference, Amsterdam, Paper No. 1151, pp. 1-3 (1996).
5. Marbach, R., and Heise, H.M., Applied Optics **34**, 610-621 (1995)
6. Reeves, III, J.B., J. AOAC 77, 814-820 (1994)
7. Reeves, III, J.B., J. Near Infrared Spectrosc. **2**, 199-212 (1994)
8. Delwiche, S.R., Norris, K.H., and Pitt, R.E., Appl. Spectrosc. **46**, 782-789 (1992)
9. Lin, J., Zhou, J., and Brown, C.W., Appl. Spectrosc. **50**, 444-448 (1996)
10. Molt, K., Cho, Y.J., J. Mol. Structure **349**, 345-348 (1995)
11. Bittner, A., Marbach, R., and Heise, H.M., J. Mol. Structure **349**, 341-344 (1995)