The International Journal Of Artificial Organs / Vol. 12 / no. 2, 1989 / pp. 129-135

Artificial Pancreas

Blood glucose measurement by infrared spectroscopy

H. ZELLER[1], P. NOVAK[2], R. LANDGRAF[3]

[1]Institut für Physikalische Medizin, Baden-Baden - West-Germany
[2]Dornier Medizintechnik GmbH, Germering - West-Germany
[3]Med. Klinik Innenstadt der Universität München, München, West-Germany

ABSTRACT: For the development of an implantable artificial endocrine pancreas, a sensor for blood glucose measurement is needed providing a long-term stability. This goal can be achieved by the application of infrared spectroscopy which, unlike electrochemical sensors, responds directly to the glucose molecule.

An investigation under physiological conditions revealed five glucose absorption bands in the near and middle infrared range. These are 1040, 1085, 1109, 1160 and 1365 cm^{-1}. Only the 1040 cm^{-1} frequency coincides with none of the other infrared-active blood substances like proteins, lipids and urea. Nevertheless, the other absorption bands too, especially the 1109 cm^{-1} frequency, can be used for blood glucose measurement, if the superimposed absorptions are compensated. Methods for the compensation have been found. Technically feasible embodiments of an infrared glucose sensor are described. (Int J Artif Organs 1989; 12:129-35)

KEY WORDS: Glucose sensor, Infrared spectroscopy, Artificial endocrine pancreas, Blood absorption

INTRODUCTION

A series of studies have shown that normoglycaemia is an absolute prerequisite to retard or arrest the development and progression of diabetic microangiopathy in the kidney, the retina, and in autonomic and peripheral nerves (1). With currently available techniques long-term normoglycaemia cannot be achieved. The artificial endocrine pancreas (Biostator^{R}), has a sensor that measures blood glucose continuously, thus permitting normal glucose control, but the instrument is large and expensive. An artificial pancreas consisting of an insulin pump, a microcomputer, light source and glucose sensor has to be so small that it can be implanted or carried by diabetic patients.

This study presents a well known but often overlooked method for blood glucose measurement, the most important prerequisite for adequate insulin replacement in diabetics: infrared spectroscopy. Previous sensors use electrochemical methods, but spectroscopic measurement does not alter the glucose molecule.

The principle of spectroscopy is as follows. A light source emits light at a given wavelength which will be absorbed by the glucose molecule. The energy exchange involves vibration of the molecule. The detector converts the transmitted radiant energy into an electrical voltage, which is processed by the microcomputer to give the appropriate signal for an insulin pump. The light source provides monochromatic infrared radiation of high energy density which is necessary for exact measurement. Only semiconductor-diode lasers are small enough and have low energy consumption using short repetitive light impulses, with a low thermal load for the tissue. Therefore high-energy diode lasers are required in any final *in vivo* blood glucose measuring system.

The aim of this study was to find wavelengths in the near and middle infrared region (0.8 — 20 μm i.e. 12500-500 cm^{-1}), where blood glucose measurement

This paper has been presented in part at the ISAO-ESAO Joint Meeting, Munich, September 1987

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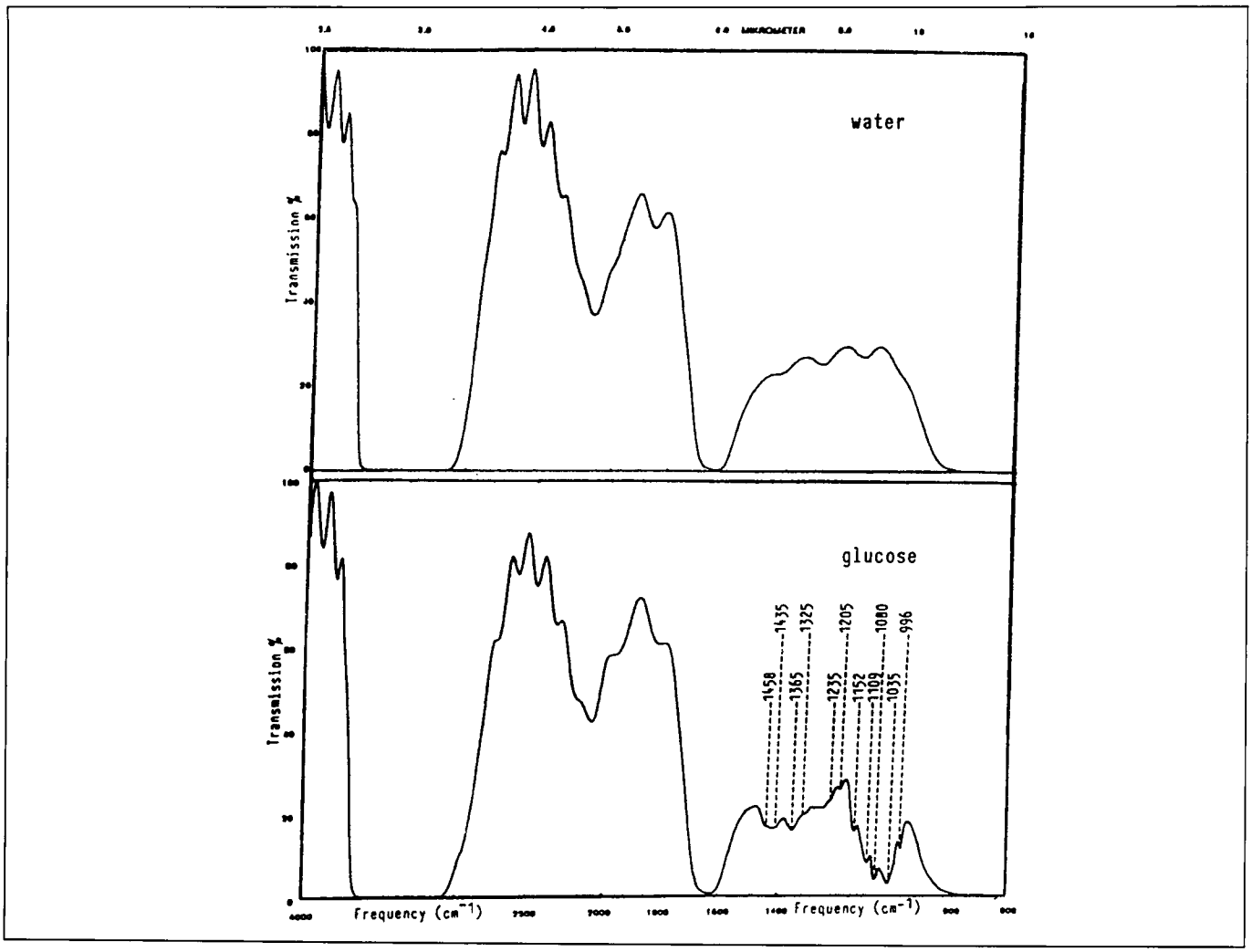

Fig. 1 - *Single-beam spectra of water (above) and aqueous glucose solution (36 g/dl) (below).*

is possible. Conventional IR spectroscopy is employed in this step since absorption of the glucose molecule has to be determined at every wavelength. The absorption of other infrared active blood substances was also investigated in relation to interference with blood glucose measurement.

MATERIALS AND METHODS

The spectra in the middle infrared (MIR) range (4000-500 cm^{-1} i.e. 2.5 — 20 μm) were recorded on a Perkin Elmer spectrophotometer model 580. A commercial dismountable liquid cell fitted with 2 mm ZnSe windows was used. ZnSe is a suitable material since it is resistant to aqueous solutions (e.g. blood), acids and alkalines. Additionally it is translucent up to 500 cm^{-1}, and shows no self-absorption. Therefore no compensation of the ZnSe windows is necessary. On account of the high refractive index of this material, an interference fringe pattern is superimposed on the absorption spectrum in the range of low absorption coefficients, when the test cell is filled with water or aqueous solutions (2). Although BaF_2 windows should be preferable, because they lose less energy and show no interference fringe pattern, their use was stopped since it was observed that blood substances are partially desulfated by exchange with the fluoride.

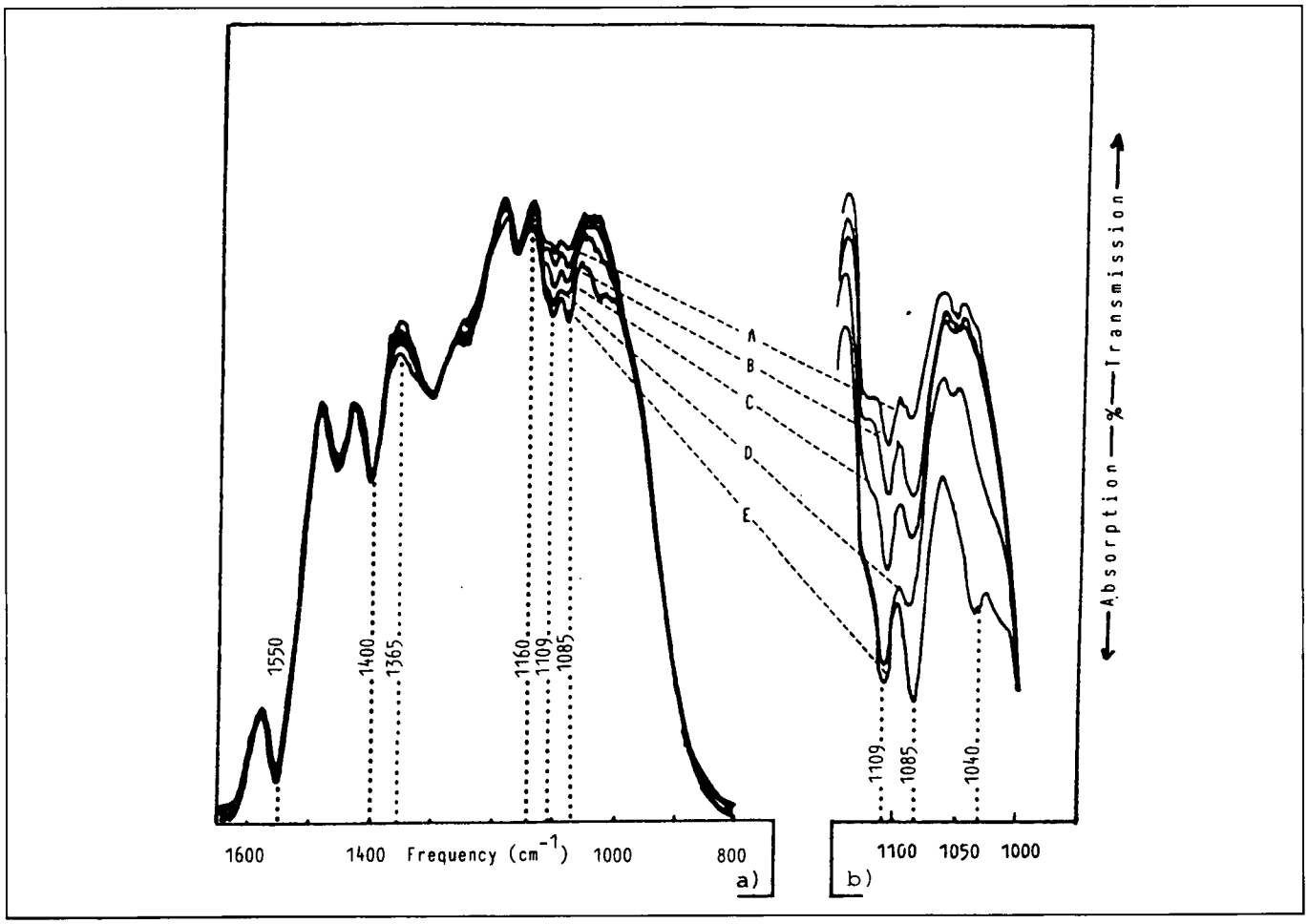

Fig. 2 - *Single-beam spectra of*
(A) blood + 80 mg/dl glucose
(B) blood + 260 mg/dl glucose
(C) blood + 440 mg/dl glucose
(D) blood + 800 mg/dl glucose
(E) blood + 1520 mg/dl glucose
with (a) x5 ordinate expansion and compared to figure 1
(b) x 20 ordinate and x2 abscissa expansion lower case.

Such windows soon become coated with a layer of $BaSO_4$.

The path length of the test cell was 25 μm in the range 4000-500 cm^{-1}.

Only human proteins and anticoagulated human blood were investigated. The proteins were obtained from Sigma Chemical Company (Munich). Absorption peaks due to anticoagulants were excluded by comparing the spectra of NaF –, EDTA –, citrated and heparin blood. With an eye to the possible application in an *in vivo* system, all spectra were recorded by the single-beam method, where the absorption of the solution (i.e. blood) is not compensated. Thus, specific absorption peaks were obtained by comparing the spectra of water, aqueous glucose and protein solutions and blood.

The abscissae of the figures are divided, as usual in infrared spectroscopy, into wave-numbers, not wavelengths. The wave-number is the reciprocal of the wave-length.

RESULTS

The absorption spectra of water and aqueous glucose solution in the MIR range are presented in Figure 1. The peaks at 3900, 2500 and 1900 cm^{-1} (water

and glucose), as well as the peaks in the range 1650-800 cm^{-1} (only water) are due to the interference fringe pattern, whose cause has been mentioned above.

Although the glucose concentration is very high, differences in these two spectra can only be seen in the range 1650-800 cm^{-1} which is called the "fingerprint region". The fine-structure absorption peaks shown in crystalline state are absent in the solution spectrum. Thus, the expected absorption of the various CH groups at 2900 cm^{-1}, and the O-H bending modes at 3320 and 3410 cm^{-1}, are totally obscured by the very intense absorption of water.

Broad glucose absorption is recorded at 1435 cm^{-1}, where a second absorption at 1458 cm^{-1} is integrated. The latter frequency is caused by bending vibrations of the CH_2 group, and the 1435 cm^{-1} of the CH group (3).

The absorptions at 1365 and 1325 cm^{-1} could be assigned to the CH_2 groups in beta- and alpha-glucose, respectively (4). Broad, very strong absorption appears in the range of 1200 - 990 cm^{-1}. The 1152 cm^{-1} frequency observed for most saccharide structures is assigned as predominantly a ring-vibrational mode with some C-O-H and C-C-H components (3). The strong peak at 1109 cm^{-1} is caused by the stretching vibrations of the endocyclic C-O-C group (4). In this range the intensity is greatest at the frequencies 1080 and 1035 cm^{-1}. The latter wave-number is due to the C-O stretching of alpha-, and the former to the C-O stretching of beta-glucose (4). In the near infrared (NIR) region the expected overtones and combination bands of the several fundamental vibrations of the glucose molecule are completely overlapped by water absorption (not shown). Thus, no glucose absorption is detectable in the NIR and MIR range in these conditions, except in the fingerprint region. Therefore the following investigations are restricted to this range.

Glucose absorption bands in blood and their different sensitivity are shown in Figure 2. The bands at 1085 and 1109 cm^{-1} show the highest sensitivity followed by the wave-numbers 1160 and 1365 cm^{-1}. The most intense absorption within the glucose spectrum at 1035 cm^{-1} can be observed only at higher glucose levels. At other glucose bands there is no increase in absorption.

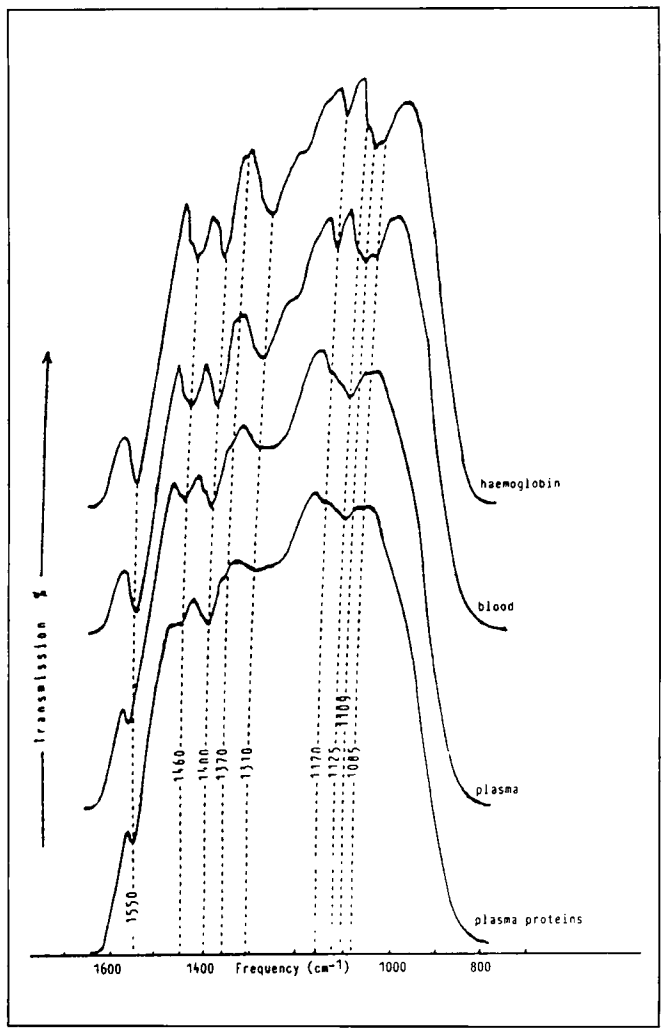

Fig. 3 - *Single-beam spectra of aqueous haemoglobin solution (16 g/dl), blood (haemoglobin, 14.7 g/dl), plasma (albumin, 4.57 g/dl, globulins, 2.5 g/dl), plasma proteins in aqueous solution (albumin, 4 g/dl, globulins, 2.5 g/dl)*

The 1400 cm^{-1} band due to the symmetrical stretching vibration of the COO^- group (5) remains unaltered as does the 1550 frequency. The latter is assigned to a C-N stretching and C-N-H bending mode of the protein backbone (5). The combination is termed Amid II absorption.

Since in biological material like blood not only glucose but also other physiological substances can change quite rapidly, glucose absorption interferences with other compounds like proteins, lipids, urea, etc. have to be ruled out. If additional absorption occurred at wave-numbers absorbed by glucose, the measurement of blood glucose would be incorrect.

TABLE 1 - ABSORPTION FREQUENCIES (cm^{-1}) OF SUBSTANCES INVESTIGATED IN THE 1650-900 cm^{-1} REGION

Haemo-globin	Albumin	α-β-Globu-lin	γ-Globulin	1m-Urea sol.	2m-Glucose sol.	Glucose in blood*	Blood	Plasma	Measurement of
				1617					urea
1550	1550	1560	1565				1550	1554	proteins
1471	1470	1470		1470			1471	1470	CH_3-
1458	**1460**	**1460**	**1460**		**1458**	**1458**	**1458**	**1459**	**CH_2- molecules**
1445	**1445**	**1445**			**1435**	**1435**	**1445**	**1445**	**CH-**
1421	1420						1421	1421	
1400	1402	1402	1415				1400	1404	
1370	1370	1370			1365	1365	1370	1370	glucose
			1336						γ-globulin
1310	1310	1310			1325		1308	1310	
					1300				
1265	1265				1252		1265		
		1250							
1240	1248		1240		1240		1242	1247	
1215	1215				1205	1215	1215		
1172	1175	1170	1170				1170	1170	
				1160		**1160**			**glucose**
					1152				
1125	1125	1125	1120				1125	1126	
1109					1109	1109	1109		haemoglobin, glucose
1089	1084	1080	1080		1080	1085	1085	1084	glucose
1056	1055				1060	1060	1060	1060	
					1035	1040			glucose
					1015				
					996	990	995		

*) The table lists only the bands that show an increase of absorption on addition of glucose

The spectra of important blood substances are depicted in Figure 3. All four spectra were recorded under the same conditions. Thus the absorbance of blood as an optically opaque fluid and plasma protein solution (optically transparent) is similar.

Absorbance at the glucose bands 1085 and 1365 cm^{-1} (shoulder) can be observed in all spectra. At 1109 cm^{-1} only haemoglobin leads to intense absorption. This could be assigned to the prosthetic group of haemoglobin, namely haem (6). Thus, all glucose bands are overlapped by absorption of blood substances, except the 1040 cm^{-1} frequency. Comparison of the spectra of haemoglobin and blood illustrates the enormous importance of haemoglobin in blood absorption. Every peak, every shoulder can be found in both of them. Thus, absorption caused only by lipids is not detectable. Comparing the spectra of plasma and protein solution shows very weak absorption in the latter at the 1460 cm^{-1} band belonging to vibrations of the CH_2 and CH_3 groups. This is due to the absence of plasma lipids, which are mainly responsible — together with the plasma proteins — for the strong absorption at this frequency in plasma.

The absorption frequencies of all substances investigated are summarized in Table 1. The absorption bands of the proteins correspond to the haemoglobin absorption, except the 1109 cm^{-1} band. Specific absorption peaks were obtained from the immunoglobulins at 1336 cm^{-1}, and urea at 1617 cm^{-1}.

DISCUSSION

Although the glucose concentration used in these experiments was quite high, its absorption was totally obscured by water absorption in the NIR and MIR range. Glucose absorption bands could be seen only in the fingerprint region. These bands are listed in Table 1 under the column "glucose solution". Nearly all absorption bands of glucose are detectable in blood (see under "glucose in blood") but only five wave-

numbers still give high enough sensitivity for the measurement of blood glucose. These are 1040, 1085, 1109, 1160, and 1365 cm^{-1}.

Only the 1040 cm^{-1} band is free of superimposed absorption of other blood constituents. In the spectrum of powdered haemoglobin too (6) no absorption is obtained at this wave-number, or in the spectra of several proteins (7). However, the carbohydrates as a large proportion of the immunoglobulins (e.g. 10.9% of IgM), glyco- and mucoproteins, might have an absorption at this frequency which is under the detection limit of the spectrophotometer employed. The use of lasers as light source improves the sensitivity of the measurement by a factor of about 100 compared with the conventional technique so that disturbances of glucose measurement could be evaluated more exactly at this frequency (8).

The glucose band at 1109 cm^{-1} is well suited for blood glucose measurement. The sensitivity is very high and the endocyclic C-O-C vibration typical for glucose. The disadvantage of coincident haemoglobin absorption could be eliminated by specific computer programs (9).

The wave-numbers 1085, 1160 and 1365 cm^{-1} are overlapped by absorption of other blood compounds. Protein absorption could be compensated, if there is a band absorbed by proteins only. Vibration of the protein backbone at 1550 cm^{-1} is very typical of the proteins. Thus, protein concentration can be determined specifically at this frequency. At 1085 cm^{-1} absorption of protein and glucose is obtained. The ratio of absorption at these two bands gives the blood glucose concentration. This method can be applied for the wave-numbers 1160 and 1365 cm^{-1} too but in this case coincident urea absorption has to be considered.

No specific absorption bands due to lipids are detectable. Lipid absorptions are observed only in the non-specific range of the several CH groups at 1365 and 1460 cm^{-1}. Their importance in blood absorption seems limited.

CONCLUSIONS

In the near and middle infrared range there are only five wave-numbers where glucose could be measured. These are 1040, 1085, 1160 and 1365 cm^{-1}. Due to interference with proteins (1085), haemoglobin (1109), urea (1160) and all CH_2 groups (1365) and their different sensitivities, the most promising wave-numbers are 1040 and 1109 cm^{-1}. Other physiological substances like haemoglobin (1109), immunoglobulins (1336), total proteins (1550) and urea (1617) are measurable with this technique.

Continuous *in vivo* blood glucose measurement is possible using transmission, reflection or attenuated total reflection (ATR) (8) methods in combination with high-energy diode lasers. Optimal location of the sensor depends on the measuring system. Thus non-invasive measurement was suggested, placing the sensor on the skin (10), oral mucosa (8) or in subcutaneous tissue. But a fixed path length between sensor and blood vessel is required, as is usual for quantitative spectroscopic measurement. Vasoconstriction and dilatation can change this distance quite rapidly, so that incorrect measurements would result.

We prefer to implant the sensor into the wall of an artery. Using the ATR-method the need for a fixed path length is eliminated because the sensor is in direct contact with blood. But soon the surface becomes coated with a layer of proteins, fibrin and platelets (11). A material must be found for the development of an artificial endocrine pancreas, which is nontoxic and nonreactive in blood.

Reprint requests to:
Dr. P. Novak
Dornier Medizintechnik GmbH
Industriestr. 15
D-8034 Germering, West-Germany

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