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ORIGINAL PAPER

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New concept for the non-invasive determination of physiological glucose concentrations using modulated laser diodes

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Abstract The detection of small absorption differences is one of the basic requirements for the non-invasive determination of blood substrates. As conventional spectrometers are limited in their detection capabilities of small absorption changes, semiconductor lasers are applied for this purpose. By using a special modulation scheme, physiological glucose concentrations could be successfully predicted. The results demonstrate the feasibility of using small and inexpensive laser diodes for the spectroscopic determination of glucose in aqueous solutions as a step towards a portable non-invasive glucose sensor for self-monitoring of diabetic patients.

Introduction

The non-invasive monitoring of blood contents would be useful for many medical applications. For example for diabetic subjects it is necessary to monitor their blood glucose-levels several times a day [1].

Also the frequent determinations of cholesterol are useful for people suffering from cardiovascular diseases [2]. The compliance of those patients for periodic determinations of their blood parameters would be greatly enhanced if these measurements could be done completely painless and non-invasive.

Infrared spectroscopy has the potential to be used for this purpose. The method has special advantages for the development of a portable device for self-monitoring of the patients since other non-invasive methods like nuclear magnetic resonance (NMR) or electron spin resonance (ESR), which have been readily used for the non-invasive monitoring of metabolic processes [3], are too costly and large-scale for a compact sensor design.

There are several approaches to the spectroscopic method. Some of the investigators have used the mid-infrared region of the electromagnetic spectrum (MIR: 4000 cm^{-1} to 400 cm^{-1}) [4, 5]. In the meantime it is widely accepted that MIR spectral analysis is of little use for non-invasive measurements as the absorption coefficient of human skin and tissue is too large for electromagnetic radiation to penetrate up to an acceptable depth into the human body [6].

Larger penetration depths are obtained within the so called therapeutic window. This spectroscopic window expands from about 600 nm to 2500 nm (16667 cm^{-1} to 4000 cm^{-1}). In this near-infrared (NIR) region the absorption coefficient of water as the main tissue constituent is low enough to allow a penetration depth up to 1 cm in human tissue [6].

The absorption bands in the NIR region correspond to relatively weak overtones and combinations of infrared fundamental vibrations of the molecules and their functional groups [7, 8]. Therefore, the spectral variations of an aqueous solution due to varying glucose concentrations are quite small. For example, even in the relative absorption maximum at 1575 nm the absorption change of an aqueous solution due to a physiological glucose concentration (100 mg/dl) was found to be in the order of 10^{-4} A.U. for 1 mm optical path length [1]. This value is in the same order of magnitude as the peak-to-peak noise level of commercially available Fourier transform spectrometers. Absorption changes due to other medically important blood substrates like cholesterol or total protein are in the same range [9, 10]. Therefore, conventional spectrometers are working very close to their limitations and various techniques like averaging, smoothing and filtering etc. have to be used to obtain acceptable data [11]. Furthermore, the application of Fourier transform and other spectroscopic techniques like scanning monochromators or optical narrow

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bandwidth filters [9] suffer from similar problems as they require high precision optics and mechanically moving parts. So they are relatively expensive and bulky devices and seem to be of little use for the development of rugged and compact sensors.

Another important point is, that the detectivity of a spectroscopic apparatus strongly depends on the spectral power density of the light source [12]. Even with high power broadband thermal sources like tungsten halogen or xenon arc lamps, power densities of only less than 1 mW/nm can be obtained. In contrast, with non-thermal laser sources power densities of several W/nm can be achieved [13]. New developments in the area of diode lasers led to compact and high power narrow-bandwidth light sources of high spectral power density. These diodes are available with several wavelengths in the NIR region. They were mainly designed for telecommunication purposes at wavelengths of 780 nm, 830 nm, 1300 nm and 1550 nm, but there are no physical limitations to these discrete wavelengths [14].

As these semiconductor diodes are very compact and reliable devices, they are well suited for the development of small and easy-to-use spectroscopic sensors.

Experimental

The experimental setup is shown in Fig. 1. Three semiconductor lasers (MRV products, USA) were used as narrowband spectral light sources. Laser *1* emitted at 834 nm, laser *2* at 1304 nm and laser *3* at 1554 nm. The maximum cw output powers were 30 mW for laser *1* and 20 mW for laser *2* and *3*, respectively. The laser diodes were operated with three laser diode drivers (PROFILE, Germany). These devices are precision current sources optimized for driving laser diodes with currents up to 200 mA. The injection currents can be modulated with arbitrary waveforms up to a frequency of 100 kHz. As modulation source the internal oscillator of the lock-in amplifier (STANFORD RESEARCH SYSTEMS, USA) was used. This reference output was connected to an electronic phase shifting unit which provided two output signals with a phase difference of 180°. The signals were fed to a multiplexer which individually distributed the signals to the modulation inputs of the laser diode drivers.

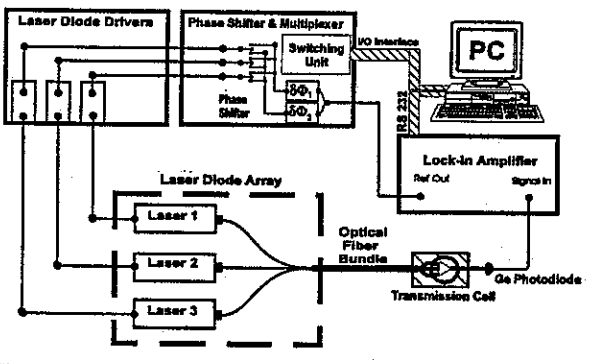

Fig. 1 Experimental set-up

The output power of the laser diodes were coupled to optical quartz fibers with core diameters of 1000 µm (Quartz & Silice, France). The three fibers were combined to a fiber bundle and directed to the 2 mm optical pathlength transmission cell. The ends of the input fibers were all located within the acceptance angle of a fourth fiber which was used to guide the transmitted beam to a Ge photodiode (EG&G, USA). The output current of this photodetector was directly fed to the current input of the lock-in amplifier.

In contrast to a conventional spectrometer this setup possesses three separate monochromatic light sources. These light sources can be modulated independently and a special modulation technique can be applied. For clarity, the principle of this method is first explained for two lasers.

The laser diodes were amplitude-modulated by modulating their injection currents with a frequency of 1.3 kHz. By using the phase shifters, the optical output signals of the laser diodes were set to a 180° phase difference. As the modulation amplitudes of each laser diode can be adjusted individually, it is possible to find a setting at which the sum of the two output powers add up to a constant value (Fig. 2a). In this case a light beam of constant power but alternating wavelength reaches the photodetector. The similar adjustment can be achieved if the light is first passed through a sample cell containing a standard solution of known analyte concentration before it reaches the detector. The wavelength dependency of the photodetector sensitivity can also be compensated by an appropriate modulation amplitude setting. If the modulation amplitudes are adjusted in this way, only a constant dc current is detected in the electrical signal of the Ge photodiode. In this case the ac dependent output signal of the lock-in amplifier is exactly zero. As the wavelength of the laser diodes are selected in a way that one diode emits a wavelength which is strongly absorbed by the analyte (analytical line: 1554 nm) and another in a spectral region where the absorption coefficient of the analyte is much lower (background wavelength: 1304 nm), small concentration changes will disturb the adjusted compensation. This means that an ac component in the photocurrent occurs. This signal can easily be detected and amplified with the high ac gain of the lock-in amplifier and since there is no ac background signal, there are no problems with saturation of input amplifiers etc. As the background absorptions are completely compensated this technique is a so-called zero crossing method, where the zero line can be set to an arbitrary

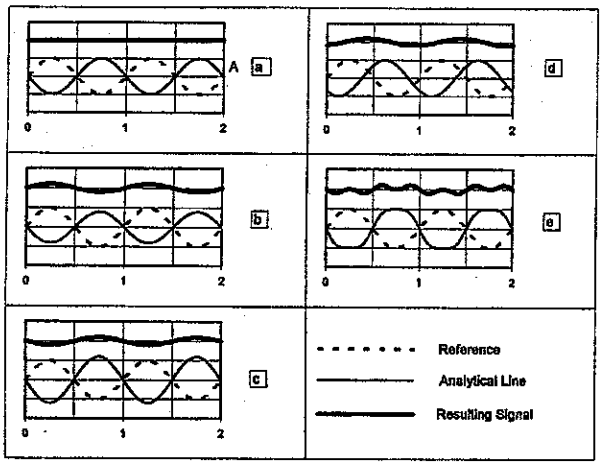

Fig. 2a–e Signal behaviour of the compensation technique. **a** Adjusted signal amplitudes. **b** Increasing analyte concentration. **c** Decreasing analyte concentration. **d** Residual signal caused by additional phase shifts. **e** Residual signal caused by non-linearities in the modulation transfer function

analyte concentration. This is particularly useful for medical applications because it is possible now to choose physiological normal values as reference points.

By using a dual phase lock-in amplifier two informations can be obtained simultaneously. The amplitude output shows the absolute value of the deviation of the analyte concentration relative to the normal value. The signal phase shows whether it is a deviation above or below this value. If for example the analyte concentration rises above the normal value, the transmission of the analytical wavelength decreases and a signal which is in phase with the background signal occurs (Fig. 2b). Correspondingly, if the analyte concentration falls below the normal value a signal with a 180° phase shift relative to the background comes up (Fig. 2c).

By choosing an appropriate phase offset, a signal function $S(r, \phi) = r \cdot \sin\phi$, with r as signal amplitude and ϕ as its phase, can be calculated. This signal function S should show a linear dependence on the absorption difference of the two wavelengths.

One way to generalize this method is to use the diodes in pairs. For N diodes $N \cdot (N-1)/2$ combinations are possible. For the distribution of the modulation signals the phase multiplexer is used. This device was designed in such a way that it is possible to connect each of the two electrical phase signals independently to each diode.

In the case of three diodes, three combinations are possible: laser *1-2*, laser *2-3* and laser *1-3*, resulting in three signals $S_{1\text{-}2}$, $S_{2\text{-}3}$ and $S_{1\text{-}3}$. For the evaluation of the signals a linear optimization method was applied to obtain a maximum correlation of the calculated output signal

$$S = S(S_{1\text{-}2}, S_{2\text{-}3}, S_{1\text{-}3})$$

$$= c_0 + k_1 S_{1\text{-}2} + k_2 S_{2\text{-}3} + k_3 S_{1\text{-}3} \quad (1)$$

and the reference glucose concentrations C.

Besides the general limitations of optical detection systems like the shot noise caused by the photon nature of light, the differential modulation method shows additional limitations. The first critical factor is the value of the phase shift between the two optical signals. Even a small additional phase shift prevents an exact compensation.

According to

$$\sin(\omega t) + \sin(\omega t + \alpha) = 2 \cos(\alpha/2) \sin(\omega t + \alpha/2) \quad (2)$$

for $\alpha \neq 180°$ a residual signal remains (Fig. 2d). For an experimental realization additional electronic phase shifts caused for example by the laser diode drivers should be kept as low as possible.

A second limiting factor are non-linearities in the transfer functions of the modulation system mostly caused by semiconductor lasers that show a non-linear light power vs injection current dependence even above their lasing thresholds.

This results in a distortion of the light modulation signal which now contains besides the main modulation frequency also higher harmonics. If these harmonics have different amplitudes, an exact compensation becomes impossible (Fig. 2e).

Another source of statistical errors are the thermal drifts of the laser diodes and of the sample. Semiconductors are strongly influenced by temperature fluctuations and laser diodes are particularly sensitive to temperature changes. First, the laser emission shifts to the long-wavelength side at a rate of about 0.2 to 1 nm/K. A second effect is, that the laser output power at constant injection current decreases with rising temperature with several percents per Kelvin [13]. As the laser diodes in our experiment were only passively temperature stabilized by a thermal isolation, temperature drifts could cause considerable errors. The absorption lines of analytes and solvents are also shifting with temperature. As the wavelength of the diodes are located at steep slopes in the water spectrum, they are therefore strongly influenced by thermal drifts. Therefore, a third laser was used. The wavelength of this diode is only weakly influenced by sample temperature variations as it is located in a flat region of the water absorption spectrum.

Results and discussion

For estimating the drift behaviour of the apparatus, a long-term experiment was carried out. For this measurement a 100 mg/dl (5.5 mmol/l) solution of D-glucose in distilled water was prepared. Then the output of the laser diodes were adjusted until a minimum output signal was obtained. The amplitude r and the phase shift ϕ was continuously monitored via the RS 232 interface of the lock-in amplifier and a personal computer for 3 h. From this data the signal function $S(r, \phi)$ was calculated. The result is illustrated in Fig. 3, which shows that the drift can reach up to 5 mV/h with averaging times of about 10 min.

The influence of the sample temperature on the signal $S_{2\text{-}3}$ is shown in Fig. 4. From this figure a temperature coefficient of 2.01 mV/K can be calculated. A closer examination of the phase behaviour confirmed that the absorption coefficient at 1304 nm increased and at 1554 nm decreased with increasing temperature. This is in accordance with the results obtained in [15, 16], showing that the absorption peaks of water are shifting towards shorter wavelengths with increasing temperature.

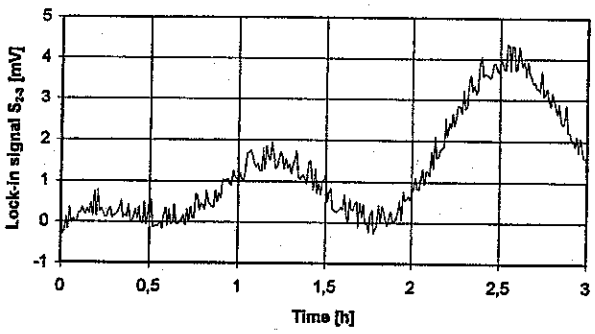

Fig. 3 Long-term drift of the output signal $S_{2\text{-}3}$

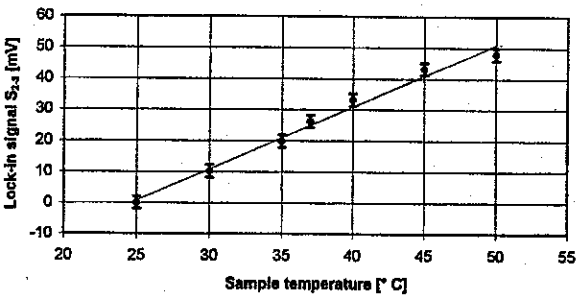

Fig. 4 Dependence of the lock-in signal $S_{2\text{-}3}$ on the sample temperature. Mean values given $\pm 1\sigma$, n = 3

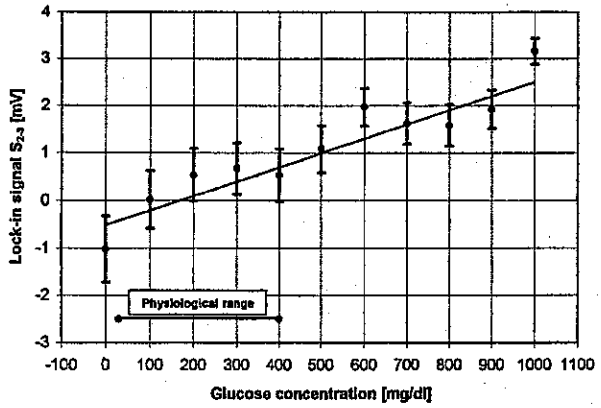

Fig. 5 Correlation between the two-wavelength signal S_{2-3} and the glucose concentration of an aqueous solution at a constant temperature of (27 ± 0.5)°C. Additionally the physiological range of glucose in whole blood of a diabetic subject is shown (30 ... 400 mg/dl; 1.6 ... 22.2 mmol/l). Mean values given with ± 1σ, n = 5

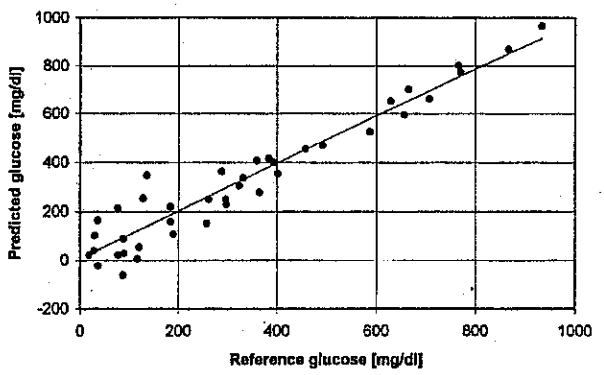

Fig. 6 Prediction of glucose concentrations using a linear optimization model. For the n = 40 values a predicted error sum of squares of $PRESS^{1/2}$ = 72.6 mg/dl was found

In Fig. 5 the dependence of the output signal on the glucose concentration by using only two lasers (#2 and #3) is shown. For this measurement the system was again adjusted to zero output with a 100 mg/dl standard solution. Then the samples with a constant temperature of (27 ± 0.5)°C and varying glucose contents ranging from 0 to 1000 mg/dl (0 to 55.5 mmol/l) were measured, each sample for n = 5 times in an arbitrary sequence. A good linear dependence of the signal S_{2-3} on the glucose concentration was found. The correlation coefficient was calculated to 0.947 and a calibration constant of 0.33 mV/(100 mg/dl) was obtained. The 1-σ-standard deviations were about 1 mV. For clarity, the physiological range of a diabetic subject of 30 to 400 mg/dl (1.67 to 22.2 mmol/l) is indicated.

To investigate the sources of the statistical errors, a calibration model with three lasers was used. Again a training set of 11 standard solutions (0, 100, 200, 300, ... 1000 mg/dl; 0, 5.5, 11.1, ... 55.5 mmol/l) was utilized to optimize the linear regression. The results for the calibration coefficients used in Eq. (1) were: $c_0 = 98$ mg/dl, $k_1 = -21$ mg/dl·mV, $k_2 = +164$ mg/dl·mV and $k_3 = +132$ mg/dl·mV.

After this, other solutions with randomly distributed concentrations were prepared and measured. Prediction of glucose by this method is illustrated in Fig. 6. A regression line according to $c_{pred} = ac_{ref} + b$ with regression coefficients $a = 0.989$ and $b = -1.2$ mg/dl was added. The correlation coefficient for the n = 40 points was calculated to r = 0.96. In addition a predicted error sum of squares $PRESS^{1/2} = 72.6$ mg/dl was found. This value still exceeds the glucose range of a healthy people of 55 mg/dl (65 to 120 mg/dl or 3.6 to 6.7 mmol/l), but the comparison of the two- and the three-wavelength method shows that the precision of the measurement can effectively be enhanced by increasing the number of laser wavelengths. Here, this is mostly due to the reduction of errors originating from sample temperature fluctuations. The remaining statistical errors are mainly due to thermally induced variations of laser output power and wavelength. By using more significant and well temperature stabilized wavelengths it will be possible to reduce the prediction error well below the physiological range of healthy people, so that an effective therapeutic control can be achieved.

Conclusions

The determination of glucose concentrations at physiological levels shows that analyte variations can be detected with acceptable precision using small and inexpensive laser diodes and a special modulation scheme. Even though the sensitivity and reproducibility of the current experimental set-up is still lower than those of commercially available invasive self-monitoring devices, they are within the range which is necessary for a threshold detector for non-physiological blood glucose values.

It was shown that the sensitivity of this method to temperature variations can be reduced by using an additional laser wavelength. Furthermore it is obvious, that with the selection of more appropriate wavelengths also other interfering effects caused by substrates with similar absorption spectra can be modelled. The combination of multivariate calibration models with a laser diode array emitting more than 10 different wavelength may lead to practicable accuracy even in a multicomponent matrix like whole blood. The efficiency of such models using continuous spectra was already demonstrated by several investigators [17–20]. The reduction of the required wavelength information by selection of lines carrying the most important

information using global optimization algorithms is also currently investigated [21, 22].

Multiwavelength semiconductor laser arrays emitting up to more than 20 different wavelength will soon be available [23]. A prototype of a multiwavelength laser unit using the differential modulation scheme is currently under investigation. The application of such devices could lead to compact and affordable spectroscopic systems which can be applied to construct small non-invasive glucose monitors for diabetic patients.

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POSTER

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Noninvasive determination of blood constituents using an array of modulated laser diodes and a photoacoustic sensor head

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Abstract Using photoacoustic laser spectroscopy, the noninvasive determination of blood constituents like hemoglobin and glucose is feasible. The aim of our investigations is the development of a sensor which is suitable for continuously noninvasive monitoring of blood glucose concentrations in diabetic patients. For this purpose a photoacoustic sensor head was developed and coupled via an optical fiber bundle to an array of 8 laser diodes emitting at various wavelengths in the near infrared region. Applying a special modulation scheme, the tiny changes of the absorption coefficient of whole blood caused by the variations of blood glucose concentrations could be measured. A resolution of 70 mg/dl was achieved, a value which is already close to the clinical requirements for a continuously working glucose sensor.

1 Introduction

The noninvasive determination of blood and tissue parameters could be one of the most valuable applications of photoacoustic laser spectroscopy (PALS). The creation of the photoacoustic signal in the sample is based on the conversion of absorbed light into heat by non-radiative processes. The heat-induced expansion generates acoustic shock waves. The waves penetrate the sample and can be detected by a pressure-sensitive element [1]. Using the Q absorption band, which is located in the region of 500 to 600 nm, an absorption spectrum of hemoglobin can be obtained noninvasively. The near-infrared spectral region (700 to 1800 nm) is well suited for the noninvasive detection of glucose, because the glucose molecule shows a significant absorption maximum at 1.57 µm. Due to spectral interferences of other blood components, a single wavelength measurement cannot provide a reliable glucose determination. The influence of the spectral interferences can be reduced by using several laser wavelengths and by applying the methods of multivariate calibration [2]. If up to 8 lasers with suitable wavelengths are selected, a single analyte like glucose can be determined even in a very complex matrix like whole blood.

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2 Experimental

Experiments were carried out using an excimer pumped dye laser as tunable laser source in the wavelength region between 520 nm and 600 nm. For the detection of the photoacoustic signal a piezoelectric ceramic was used. The ceramic was integrated into a photoacoustic sensor head which was coupled to the dye laser via an optical fiber. The experimental setup was mainly used for the noninvasive measurement of hemoglobin spectra and the determination of the depth-resolving capabilities of the photoacoustic principle [3].

For measurement in the near-infrared region, a laser diode assay was developed (Fig. 1). For the detection of the photoacoustic signal, a lock-in amplifier was used. In order to increase the sensitivity of the system, a special modulation scheme was applied [4].

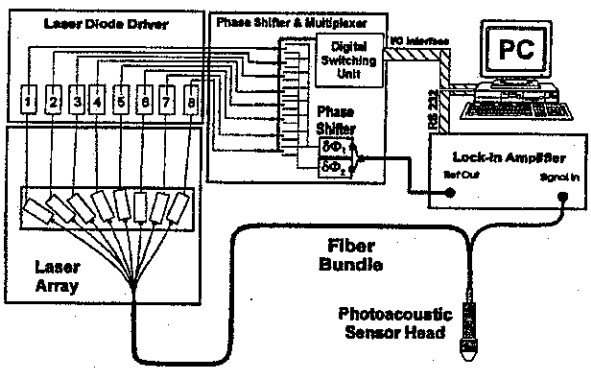

Fig. 1 Laser diode array with 8 modulated semiconductor lasers

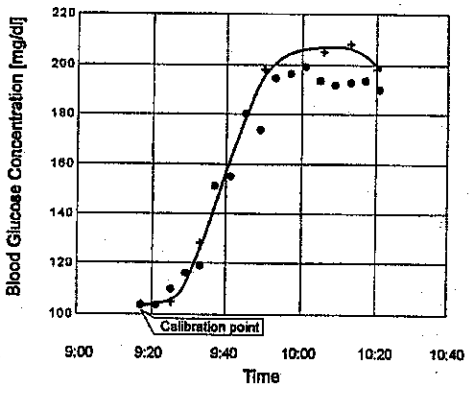

Fig. 2 Blood glucose values during a tolerance test with points determined by a clinical enzymatic glucose analyzer (+) and by the photoacoustic sensor system (●)

3 Results

The in vitro photoacoustic spectra of whole blood taken through a highly light scattering vinyl membrane showed good agreement with reference transmission spectra of diluted hemoglobin solutions which were obtained with a commercial spectral photometer. In vivo spectra taken from various parts of the human body also showed the two absorption peaks of oxyhemoglobin. These results confirmed that the photoacoustic spectroscopy is an effective method for obtaining blood spectra in a noninvasive way.

For the determination of glucose, a diode laser array was used. The wavelengths of the diodes were selected in a way that the resulting calibration showed a minimum prediction error. With this arrangement, an oral glucose tolerance test was carried out with several test persons. The results are indicated in Fig. 2.

4 Conclusions

Results obtained with a pulsed dye laser system have shown that photoacoustic laser spectroscopy is well suited for the noninvasive determination of blood spectra.

The determination of glucose concentrations at physiological levels in a model tissue shows that variations of glucose levels can be monitored with acceptable precision using small and inexpensive laser diodes and a special modulation scheme. High-integrated multiwavelength semiconductor laser arrays emitting at several wavelengths will soon be available. Such devices could lead to compact and affordable spectroscopic systems which can be applied for the development of small noninvasive glucose monitors for diabetic patients.

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