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(54) Title: METHOD AND APPARATUS FOR NON-INVASIVE DETECTION OF PHYSIOLOGICAL CHEMICALS, PARTICULARLY GLUCOSE

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│   IRRADIATE PATIENT WITH NIR RADIATION   │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│   COLLECT DATA CONCERNING TRANSMITTED    │
│      OR REFLECTED NIR RADIATION          │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│    DIGITALLY FILTER COLLECTED DATA TO    │
│       ISOLATE GLUCOSE INFORMATION        │
└─────────────────────────────────────────┘
                    │
                    ▼
┌─────────────────────────────────────────┐
│  APPLY MATHEMATICAL MODEL TO FILTERED    │
│  DATA TO DETERMINE GLUCOSE LEVEL IN      │
│  PATIENT                                 │
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(57) Abstract

    Non-invasive measurements of physiological chemicals such as glucose are made using infrared radiation and a signal processing system that permits the construction of a device suited for home use. The level of a selected physiological chemical in a test subject is determined in a non-invasive and quantitative manner by a method comprising the steps of: (a) irradiating a portion of the test subject with near-infrared radiation such that the radiation is transmitted through or reflected from the test subject; (b) collecting data concerning the transmitted or reflected infrared radiation using a detector; (c) digitally filtering the collected data to isolate a portion of the data indicative of the physiological chemical; and (d) determining the amount of physiological chemical in the test subject by applying a defined mathematical model to the digitally filtered data. The data collected may be in the form of an absorbance spectrum, i.e., a representation of the variation in absorbance as a function of wavelength; a single beam spectrum or in the form of an interferogram, i.e., a representation of infrared light intensity as a function of the retardation of the moving mirror in the interferometer, and thus of time.

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METHOD AND APPARATUS FOR NON-INVASIVE DETECTION OF PHYSIOLOGICAL CHEMICALS, PARTICULARLY GLUCOSE

BACKGROUND OF THE INVENTION

This application relates to a method and apparatus for the non-invasive, quantitative measurement of physiological chemicals, particularly glucose, in a
10 patient.

Determination of blood glucose is a routine proce-dure performed several times a day by many diabetics. In general, this procedure involves the taking of a small blood sample and evaluating the level of glucose in the
15 sample. Common instruments used for this purpose use the enzyme glucose oxidase to convert glucose and oxygen to gluconic acid and hydrogen peroxide, and then measure the level of peroxide by either spectroscopic or electrochemical means.

20   While these daily measurements provide the diabetic patient with the ability to self-monitor and thus better control blood glucose levels, they are not without draw-backs. In particular, the taking of blood samples several times a day can be painful and exposes the patient to a
25 risk of infection. Moreover, using this existing technology it is impossible to obtain a continuous blood glucose measurement. Thus, during the night, a patient must either be awakened periodically for testing or run the risk that glucose levels will drop to dangerous levels
30 as they sleep.

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In view of the foregoing, there exists a real need
for a non-invasive method of measuring blood glucose in a
patient. It has been suggested that this could be accom-
plished using near-infrared (NIR) radiation. Thus, U.S.
5   Patent No. 5,086,229 of Rosenthal, which is incorporated
herein by reference, describes a system using a plurality
of infrared light emitting diodes and a detector to
measure blood glucose. The infrared measurement of
glucose in the body is immensely complicated, however,
10 because of the substantial absorbances of fats and
proteins in the near-infrared. Thus, while the Rosenthal
patent presents an interesting theory, there is no
published documentation that demonstrates the ability of
the Rosenthal system to measure glucose noninvasively.
15 Furthermore, the wavelengths used in the Rosenthal system
have never been shown to possess useful glucose
information.

It is an object of the present invention to provide
a practically useful non-invasive near-infrared detector
20 for blood glucose and other physiological chemicals that
is adaptable for continuous use.

It is a further object of the present invention to
provide a method for the non-invasive quantitative
measurement of physiological chemicals and particularly
25 glucose in a patient that can be used either continuously
or intermittently.

It is still a further object of the invention to
provide an apparatus for continuous or intermittent non-
invasive monitoring of blood glucose and other
30 physiological chemicals in a patient.

SUMMARY OF THE INVENTION

In accordance with the invention, non-invasive
measurements of physiological chemicals such as glucose

are made using infrared radiation and a signal processing
system that permits the construction of a device suited
for home use. Thus, the level of a selected physiological
chemical in a test subject is determined in a non-invasive
and quantitative manner by a method comprising the steps
of:

       (a)   irradiating a portion of the test subject with
near-infrared radiation such that the radiation is
transmitted through or reflected from the test subject;

       (b)   collecting data concerning the transmitted or
reflected infrared radiation using a detector;

       (c)   digitally filtering the collected data to
isolate a portion of the data indicative of the
physiological chemical; and

       (d)   determining the amount of physiological
chemical in the test subject by applying a defined mathe-
matical model to the digitally filtered data. The data
collected may be in the form of an absorbance spectrum,
i.e., a representation of the variation in absorbance as a
function of wavelength; or in the form of an interfero-
gram, i.e., a representation of infrared light intensity
as a function of the retardation of the moving mirror in
the interferometer, and thus of time.

       In a further aspect of the invention, there is
provided a device for measurement of a chemical in a
sample comprising:

       (a)   means for collecting data concerning near-
infrared radiation transmitted through or reflected from
the sample;

       (b)   means for digitally filtering the collected
data to isolate a portion of data indicative of the
chemical;

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(c) means for applying a defined mathematical model
to the digitally filtered data, whereby the amount of
chemical in the test subject is determined; and

(d) means for reporting the amount of chemical in
5   the sample. This device can be incorporated into a
complete apparatus for the non-invasive measurement of
blood glucose. Such an apparatus would also include a
source of infrared radiation and a mechanism for directing
the infrared radiation from the source to the test subject
10 and from the test subject to the means for collecting the
spectrum.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a flow chart depicting the method of the
invention;
15   Fig. 2 is a flow chart depicting a process for
digitally filtering an absorbance spectrum;

Fig. 3 is a schematic representation of an
interferometer;

Fig. 4 shows a sample interferogram;
20   Fig. 5 shows a device in accordance with the present
invention for measurement of glucose in a sample;

Fig. 6 shows an apparatus in accordance with the
present invention for non-invasive monitoring of
physiological chemicals in a test subject;
25   Fig. 7 shows unfiltered interferogram segments taken
of phosphate buffer and three concentrations of glucose;

Fig. 8 shows digitally filtered interferogram
segments taken of phosphate buffer and three
concentrations of glucose;
30   Fig. 9 shows the relationship between filtered
interferogram segment magnitude and actual glucose
concentration;

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Fig. 10 shows the relationship between glucose concentration determined from digitally filtered absorbance spectra of human test subjects and actual glucose levels as determined by blood testing;

5   Fig. 11 shows the relationship between glucose concentration determined from unfiltered absorbance spectra of human test subjects and actual glucose levels as determined by blood testing;

Fig. 12 displays the data obtained from applying
10   three distinct filters to an interferogram obtained from a glucose/BSA/triacetin sample;

Fig. 13 plots the standard error of calibration (solid line) and prediction (dashed line) versus the number of PLS factors used to form the calibration model
15   of a filtered interferogram in a two-filter analysis based on interferogram points 340-640 for the glucose filter and points 200-301 for the BSA filter;

Fig. 14 is a correlation plot of glucose concentrations estimated using a 14 term calibration model
20   versus the corresponding actual concentrations;

Fig. 15 is a correlation plot of glucose concentrations estimated using a 15 term calibration model for an unfiltered interferogram versus the corresponding actual concentrations;

25   DETAILED DESCRIPTION OF THE INVENTION

Fig. 1 is a flow chart depicting the method of the invention, as applied to the non-invasive determination of blood glucose in a human patient. As shown, the first step of the method is the irradiation of the test subject, in
30   this case the patient, with near-infrared radiation.

Suitable NIR radiation for use in the present invention coincides with the absorbance bands of glucose or other physiological chemical being measured. For

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glucose, these bands are located in the regions of 5000-4000 cm^{-1} and 6500-5800 cm^{-1}. The intensity of the light at the selected wavelength should be on the order of 700 lux or greater. Such radiation can be produced by a 300 W

5 tungsten-halogen lamp.

Since the spectrum obtained and used in the method of the present invention may be either a transmittance spectrum or a reflectance spectrum, considerable latitude is available in the manner and location in which the NIR

10 radiation impinges on the test subject. For example, if transmitted NIR radiation is being measured, the NIR radiation should impinge on a relatively thin, fleshy area of the patient such as the fleshy webs between the fingers or toes or the ear lobe. If reflectance spectra are to be

15 used, the sampling site should be characterized by high blood flow close to the surface, such as the ventral surface of the wrist.

The source of NIR radiation used in the present invention may be such that it is disposed directly against

20 the surface of the test subject. For example, a small halogen lamp could be used. Alternatively, the source may be physically remote from the test subject. In the latter case, it is advantageous, although not necessary, to guide the NIR radiation to the desired irradiation site on the

25 surface of the test subject, for example by means of optical fibers.

In the second step of the method, the data concerning the transmitted or reflected NIR radiation is collected using a detector. The specific nature of the

30 detector is not critical, provided it is capable of detecting the pertinent wavelengths of light and responding rapidly enough to be compatible with the other components of the device. An example of a suitable detector for collection of an absorbance spectrum is a combination of a

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dispersive element, e.g., a grating or prism, and an
optical multi-channel analyzer sensitive to NIR radiation.
In the case where the data is to be collected as a single
beam spectrum, an absorbance spectrum or an interferogram,
5   a suitable detector is a combination of a NIR interfero-
meters and a photon counting detector such as a solid
state indium antimonide detector.

The positioning of the detector relative to the test
subject will depend both on the nature and size of the
10   detector and the environment in which the measurement is
being taken. For most purposes, it will be desirable to
have the detector physically separated from the test
subject, both because of detector size and to maximize
detector performance by providing the detector with a
15   stable environment. It will therefore generally be
advantageous to guide the transmitted or reflected NIR
radiation to the detector, for example using optical
fibers.

Depending on the instrumentation selected, the data
20   concerning the transmitted or reflected radiation is
collected as either an absorbance spectrum or an
interferogram. In either case, the next step as shown in
Fig. 1 is to digitally filter the data to isolate the
portion of the data which is indicative of the chemical of
25   interest. The specific manner in which this step is
performed depends on the form of the collected data.

Digital Filtering of an Absorbance Spectrum
When the data collected is in the form of an
absorbance spectrum, the process for filtering the data is
30   shown in Fig. 2. As shown, the absorbance spectrum, or a
portion thereof containing information about the chemical
being tested for, is transformed using a Fourier transform
into a Fourier domain spectrum

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The transformed spectrum is then filtered by multiplying it by a Gaussian function that weights spectral information associated with the chemical being analyzed. In the case of glucose, a suitable Gaussian function is

5   defined by a mean position of 0.037 f (digital frequency units) and a standard deviation width of 0.011 f. This function emphasizes a glucose-associated absorbance band appearing in the spectral region between 6500 and 5800 cm^{-1}. Other peaks, and other Gaussian functions for the same

10   peak may also be employed. For example, the glucose-associated absorbance band at 4400 cm^{-1} can be emphasized. For physiological chemicals other than glucose, appropriate Gaussian functions may be identified. For absorbance spectra processing, the proper Gaussian

15   function can be obtained by analyzing the shapes of the absorbance bands for the compound of interest.
After multiplication of the spectrum with the Gaussian function, the resulting filtered spectrum is converted back to an absorbance spectrum using an inverse Fourier

20   transform.

While the use of a Fourier filtering process such as that described above is a preferred method of performing the method of the invention, other digital filtering methods may also be used. Such methods include finite

25   impulse response and infinite impulse response digital filters.

Digital Filtering of a Single Beam Spectrum or an
Interferogram

An important aspect of non-invasive measurement of

30   glucose and other physiological chemicals using interferograms is the ability to analyze this data in the absence of a contemporaneously collected background or reference spectra. This is the case because no convenient method

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exists to collect a background spectrum such as the
spectrum of a particular human subject without glucose.

When the data concerning the transmitted or
reflected infrared radiation is collected in the form of

5   an interferogram, it may be processed in either of two
general ways. First, the interferogram may be converted
to a conventional single beam spectrum using a Fourier
transform and then filtered by multiplication with an
appropriate Gaussian function. For example, in the case

10   of a non-invasive determination of glucose, a Gaussian
function having half-power points of 4445.4 and 4354.6 cm^{-1}
can be used to emphasize the glucose absorbance band at
4400 cm^{-1}. This corresponds to a Gaussian function having
a mean position of 0.139 f and a standard deviation of

15   0.00122 f. Other Gaussian functions for digital filtering
to obtain information from other glucose absorbances or
for absorption bands associated with other chemicals of
interest can be obtained by matching functions to the
width and location of the absorption bands.

20   This filtering differs from the process described
above, in that ratioing to eliminate the background signal
is not performed. Because of this, after multiplication
of the transformed spectrum, the resulting filtered
spectrum can be subjected to an inverse Fourier

25   transformation to yield a filtered interferogram. Such an
interferogram is much simpler in appearance than the
original interferogram, because much of the noise and
absorbances not associated with the chemical of interest
have been removed. Further, there is a clear correlation

30   between the magnitude of the interferogram signal and the
amount of the chemical being measured in the test subject.

The magnitude of the interferogram peaks is advan-
tageously measured by the simple procedure of integrating
the interferogram to determine the total power of the

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signal. This yields a single value related to the glucose
concentration. Alternatively, the individual points in
the filtered interferogram could be submitted to a
multivariate calibration method such as a partial least

5   squares regression. This would yield a multivariate model
relating the individual interferogram point intensities to
glucose concentration.

Alternatively to transforming the interferogram into
a single beam spectrum, the interferogram itself may be

10 subjected to a digital filtering process, and the
magnitude of the filtered interferogram peaks analyzed
using a predefined mathematical model. We have defined a
model which predicts an approximately linear relationship
between analyte concentration and filtered interferogram

15 intensity as given by the formula

$$C \approx NP$$

where C is the concentration, P is the magnitude of the
filtered segment, and N is a sensitivity term defined as

$$N = \frac{1}{\sqrt{\sum_{k=n_1}^{n_2} Z[k]^2}}$$

where $Z[k]$ is an interferogram domain function. The
approximate linear relationship between the analyte

20 concentration and magnitude of the filtered interferogram
segment is based on the assumption that a MacLaurin series
approximation used in the derivation of the relationship
is valid. This approximation only holds for small
absorbance values. For example, Sparks et al., *Anal.*

25 *Chem.* 54: 1922-1926 (1982) have estimated that this

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approximation has less than 5% error if the absorbance value is less than 0.12. For glucose analysis using the present invention, peak absorbance of the glucose band is on the order of 10^{-4} or less. Thus, the use of this

5   approximation appears valid.

The approximate linear relationship also depends on the value of N being substantially constant across the calibration samples. This requires that the sample pathlength, molar absorptivity and the intensity of the

10   filtered reference spectrum be constant with respect to concentration.

Variation in sample pathlength is not a problem in laboratory measurements due to the use of a fixed pathlength sample cell. It could present more of a

15   problem in actual noninvasive tests performed on body tissue. Moreover, since the entire spectral band is isolated by the bandpass filter rather than a single wavenumber, some change in molar absorptivity might be expected across the concentration range. These two

20   factors could lead to limitations on the dynamic range over which the linear relationship would hold, potentially making several calibration factors necessary. They should not, however, prevent the use of the linear approximation for making noninvasive glucose determinations.

25   The assumption of constant intensity of the filtered reference spectrum is problematical, as some variation in infrared intensity is common in a Fourier transform infrared experiment due to changes over time in instrumental characteristics such as interferometer

30   alignment and detector temperature. These factors can be overcome through the use of a normalization step.

Finally, the linear relationship set forth above assumes that only a single analyte band has been isolated by the bandpass filter. If other analyte bands or bands

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from other constituents in the sample matrix fall within
the filter bandpass, their interferogram signatures will
add to the signature of the targeted analyte band. The
filtered interferogram will then possess a more complex
5   structure and the simple univariate linear relationship
will not apply. The selection of an appropriate bandpass
filter is therefore quite important to the success of the
direct measurement of interferogram intensity as a method
for noninvasive determination of physiological chemicals.
10 If suitable filters cannot be identified for a given
analyte and sample matrix, however, a multivariate
analysis procedure can be used on place of the linear
approximation as discussed in Examples 4 and 5 herein.

        Application of digital filtering directly to the
15 interferogram depends on the fact that each wavelength of
light is represented in the interferogram by a modulated
sine wave whose frequency (in hertz) depends on the wave-
length of the light and the interferometer mirror velocity
in accordance with the equation

$$\frac{frequency}{of\,signal} = 2 \times \frac{wavenumber}{of\,radiation} \times \frac{mirror}{velocity}$$

20 where the wavenumber has units of cm^{-1}, the mirror velocity
is expressed in units of cm/sec. By selecting an
electronic filter that selects out only the portion of the
interferogram having the frequency defined by this
equation for the selected absorbance of the chemical being
25 measured, a simplified interferogram is obtained.

        The construction of electronic filters to select for
specified wavelengths is commonplace in the electronic
arts. Briefly, the filter would be implemented as a
digital filter. The software controlling the device would
30 implement the filter as part of the data processing. The

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digital filter used implements an approximation of the
convolution sum

$$y[n] = \sum_{k=-\infty}^{\infty} x[k]h[n-k]$$

where y[n] is the filtered interferogram at point n, x[k]
is the original unfiltered interferogram at point k, and
5   h[n-k] are points on the impulse response of the digital
filter, i.e., the interferogram-domain representation of
the frequency response filter.

While the digital filtering procedure for interfero-
grams has been described above in terms of a Fourier
10 filtering process, other digital filtering methods may
also be used. In particular, methods such as finite
impulse response and infinite impulse response filters can
be used. These filter design methods approximate the
infinite summation in the convolution sum above. These
15 methods offer the advantage of reducing the size of the
interferogram required, which can lower the performance
requirements of the interferometer.

While the method of the invention can be practiced
on complete interferograms, it has been found that short
20 segments of an interferogram can be used as well. This
has great significance to the application of this
technology in relatively rigorous environments, because it
facilitates the construction of a smaller interferometer
with less stringent mechanical tolerances.

25   An interferometer such as that shown in Fig. 3
includes a 50:50 beam splitter 30, two mirrors 31 and 32,
and optical track 33 and a detector 34. Infrared
radiation is passed through the beam splitter 30 and

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directed toward the two mirrors 31 and 32, oriented at 90

degrees to one another, which reflect it back towards the

detector 34. At the same time, the mirror 31 is moved

back and forth on the optical track 33. The movement of

5   the mirror 31 results in successive occurrences of

constructive and destructive interference. Thus, the

signal received at the detector is a harmonic signal whose

frequency is determined by the frequency of incident light

and the mirror velocity. A sample of the detected signal,

10   called an interferogram, is shown in Fig. 4.

Interferograms can be converted by a mathematical

transform into conventional spectra of very high quality.

However, the mechanical complexity of the interferometer,

and particularly the need for precise control of the move-

15   ment of the mirror over a substantial length of travel and

for precise optical alignment, limits the applicability of

interferometers in rigorous environments. The mechanical

complexity of the instrument could be substantially

reduced if the length of the interferogram were shortened,

20   because the length of mirror travel could be shorter. It

is generally recognized, however, that the use of short

interferograms significantly reduces the quality of the

spectrum which can be obtained upon transformation of the

interferogram to a conventional absorbance spectrum.

25   The reason for this loss of quality has to do with

the mathematical approximation normally used to perform

the transformation, the fast Fourier transform or FFT.

The FFT is an approximation of the infinite Fourier

integral in which it is assumed that the time signal is

30   zero outside the region actually sampled.   Supplying a

very short interferogram to the FFT has the effect of

convolving a boxcar function with the true (i.e.

infinitely sampled) interferogram. The Fourier transform

of the boxcar function is a $(\sin x)/x$ function. When this

function is superimposed on the true spectrum, a lowering
of resolution results.

In conventional interferometers in use today, the
total travel of the mirror is around 2 cm, with spectral
5   collection actually occurring over approximately 1 cm of
this length. It has surprisingly been found, however,
that short interferogram segments, i.e., interferograms
collected over distance of mirror travel of about 0.003 to
0.10 cm can be used effectively in the method of the
10   present invention. This opens the door to the
construction of interferometers that are smaller and
inherently more rugged than their laboratory predecessors,
and which thus are suited for use, e.g., by an individual
diabetic in the home.

15   A further advantage of the method as of the present
invention is ability to dispense with separate background
spectral measurements through the use of interferograms.
Such measurements are necessary in most systems to correct
for instrumental variations and the absorbance of com-
20   pounds other than the compound of interest. In the case
of non-invasive monitoring of physiological chemicals,
however, this background is highly complex. Furthermore,
it is not readily apparent how one would obtain a spectrum
of the test subject, minus the chemical being analyzed.
25   When using interferograms, however, no background is
necessary. This is the case, because in an interferogram,
the portion of the signal which can be attributed to the
overall instrument function is a wide feature which is
represented in the interferogram as a rapidly damping
30   sinusoidal signal. In contrast, absorption bands of
compounds are narrower and thus their interferogram repre-
sentation does not damp as rapidly. Thus, by taking the
analyzed data from an interferogram segment removed from
the centerburst, the instrumentation dependent signal has

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essentially died out, leaving only the signals associated with actual infrared absorbances.

Regardless of the form of the collected data, the final step in the method as shown in Fig. 1 is the

5   determination of glucose or other physiological chemical concentration by application of a previously determined mathematical (calibration) model. In either case, the input into the model is the filtered data, and the output is the estimated concentration of glucose or other

10   physiological chemical of interest. The model is developed based upon a multivariate regression analysis, such as partial least squares (PLS) regression. In PLS, the independent variables are the filtered data and the dependent variable is the glucose concentration. Other

15   multivariate regression methods such as principal components regression can also be used.

The mathematical model resulting from application of any of these techniques to a set of data in which the actual glucose concentrations are known can be expressed

20   as a series of terms the value of which correspond to measured properties of the data for a sample, and factors or regression coefficients. For example, a model found to be useful in determining glucose concentrations in vivo using data in absorbance spectrum form has the form

$$c_i = b_0 + b_1 x_{i,1} + \ldots + b_h x_{i,h}$$

25   where c_i is the predicted glucose concentration corresponding to spectrum i, the x_i values are the partial least-squares factor scores computed from the absorbance data, and the b terms are the coefficients determined from a multiple linear regression analysis of the measured

30   glucose concentrations corresponding to the calibration

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absorbance data and the h sets of partial least-squares
scores computed from the calibration absorbance data.

To analyze an unknown sample, values measured for
the sample are substituted into this equation to produce
5   an estimate of the glucose concentration in the sample.
As a general rule, device limitations will probably
require that this model be established for each chemical
being measured using instrumentation separate from the
device actually being used by a test subject to perform
10   day-to-day monitoring. Nevertheless, some calibration
function may be included in an individual device to adapt
it to a specific test subject.

While the method of the invention has been
described principally with respect to the monitoring of
15   glucose in a human subject, the scope of the invention is
not so limited. Physiological chemicals other than glu-
cose, such as urea, lactate, triglycerides, total protein
and cholesterol may also be quantitatively detected using
the method of the invention through the selection of
20   appropriate digital filtering parameters. Furthermore,
because the filtering is all post-collection processing
performed on stored data, a single spectrum can be anal-
yzed numerous times to provide analytical results on more
than one physiological chemical.

25   Test subjects to which the method of the invention
may be applied include not only human subjects, but also
animal subjects, e.g., during veterinary procedures, and
even living plants. For example, non-invasive determina-
tion of sugar levels within fruits could be made to assess
30   the ripeness of the fruit and its readiness for harvest-
ing. Thus, as used in the specification and claims here-
of, the term test subject refers to any living organism
containing physiological chemicals.

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A further aspect of the present invention is a
device for measurement of glucose in a sample as shown in
Fig. 5. This device, which can be used on any type of
sample, not just a living organism, includes means for
5   collecting a transmitted or reflected infrared radiation
such as interferometer 50 or a combination of a dispersive
element and an optical multichannel analyzer. The
collected spectral data is transferred to a computer read-
able storage medium 51 from whence it is read by computer
10   processor 52 and processed in accordance with a programmed
instruction to digitally filter the collected spectrum to
isolate a portion of the spectrum indicative of glucose.
Suitable computer processors for use in this application
include Intel 486 and Pentium® microprocessors or MIPS
15   R3000 and R4000 processors of the type used in Silicon
Graphics workstations.

The computer program used to perform the digital
filtering steps can be written in any programming language
capable of performing a Fourier transform and multiplying
20   a data set by a Gaussian function. For example, a program
written in Fortran 77 was employed to perform the data
analysis described in the example herein.

Once the stored data have been digitally filtered,
the computer processor 52 is operated under a second set
25   of programmed instructions in order to apply a predeter-
mined mathematical model to the data. The result of this
application is a numeric estimate for the concentration of
glucose or other chemical of interest in the test subject.

The device of the invention also includes one or
30   more means for reporting the amount of glucose in the
sample. Examples of means for reporting the amount of
glucose include digital display panels 53, transportable
read/write magnetic media such as computer disks and tapes
which can be transported to and read on another machine,

and printers such as thermal, laser or ink-jet printers
for the production of a printed report.

A further aspect of the present invention is an
apparatus for the non-invasive, quantitative detection of
5 physiological chemicals in living organisms. Such an
apparatus may be a combination of a device for glucose
measurement as described above, or a comparable device
having the digital filter parameters and standard values
targeted for a different physiological chemical with a
10 means for irradiating the surface of the living organism
with near-infrared radiation in such a way that the
spectral data can be collected in a transmittance, diffuse
reflectance or transflectance configuration.

Fig. 6A shows an apparatus of this type for collect-
15 ing spectral information. The apparatus has a housing
which encloses a light source 61, a detector 62, and a
microprocessor 63. Infrared radiation from light source
61 is transmitted through optical fiber 64 to the test
subject 65. Transmitted radiation is collected through
20 optical fiber 66 and transmitted to detector 62. The
signal from the detector is then transmitted to micropro-
cessor 63. Microprocessor 63 digitally filters the signal
from the detector, and applies the predefined mathematical
model to the filtered data to determine a value for glu-
25 cose concentration which is displayed on display 67.
Display 67 may be an LED or LCD display.

Other configurations of the apparatus can be util-
ized in addition to the specific configuration shown in
Fig. 6A. For example, the apparatus can be configured to
30 collect infrared radiation in a diffuse reflectance mode,
rather than in a transmission mode. In the diffuse
reflectance mode, a portion of the incident radiation
penetrates a short distance into the sample before being
scattered out of the sample. A fraction of this back-

- 20 -

scattered radiation is collected and measured by the detector.

The apparatus may also be operated in a transflec-
tance mode. In a transflectance configuration, optical
5   fibers 64 and 66 are positioned apart by a distance D as
shown in Fig. 6B. The second fiber collects light that
has entered the sample and traversed a short distance
within the sample medium.

EXAMPLE 1

10   *In vitro* measurements of glucose concentration were
made on a series of thirteen glucose samples having
concentrations varying from 1.25 to 19.7 mM glucose in pH
7.4 phosphate buffer (0.1 M). The samples were placed in
1 mm pathlength cuvettes constructed from infrared quartz
15   and transmittance interferograms of each were obtained
using a Nicolet 740 spectrometer in conjunction with a
tungsten-halogen light source and a cryogenically cooled
InSb near-infrared detector. Double sided interferograms
were collected by averaging across 256 co-added scans.
20   Either two or three replicate interferograms were made for
each sample, producing a total of 38 interferograms across
the 13 samples.

To evaluate glucose concentration, the glucose
absorption located at 4400 cm^{-1} was used and isolated by
25   digital filtering. The filtering process involved
application of a fast Fourier transform to the
interferogram to produce a single beam spectrum;
multiplication of the transformed interferogram with a
Gaussian function having half-power points at 4445.6 and
30   4354.6 cm^{-1}; and application of the inverse fast Fourier
transform to obtain the filtered interferogram.

Fig 7 shows four interferograms segments (points
600-800 relative to the interferogram peak maximum or

- 21 -

centerburst) obtained for phosphate buffer, 3.22 mM
glucose, 7.95 mM glucose and 19.7 mM glucose. No obvious
information relating to glucose concentration can be
observed. Fig. 8 shows these same interferograms after
5   the digital filtering procedure described above. The
waveforms have been greatly simplified due to the removal
of all frequencies outside the bandpass of the Gaussian
function. The interferogram segment for the buffer de-
creases in intensity to a minimum point or node, followed
10   by a slowly damping signal due to the water absorption
feature. The interferograms of samples containing glucose
exhibit a less obvious node, but increased magnitudes are
observed with increasing glucose concentrations.

Fig. 9 shows the results obtained when the magni-
15   tudes of the filtered interferograms are plotted as a
function of actual glucose concentration. An excellent
correlation between the magnitude and the concentration is
observed (Univariate linear regression: R squared=99.0%;
standard error of calibration = 0.62 mM).

20   EXAMPLE 2

In vivo experiments on the non-invasive detection of
glucose were performed using a Nicolet 740 research grade
spectrometer configured with a 250 W tungsten halogen
lamp, CaF_2 beam splitter and InSb detector. Non-invasive
25   measurements in accordance with the invention were taken
at the same time as conventional invasive measurements on
three individuals of varied physiognomy during the course
of glucose tolerance tests. The beam of light from the
spectrometer having a spectral range from 7000 to 5000 cm^{-1}
30   was passed through a portion of the webbing between the
thumb and forefinger This particular wavenumber region
was isolated using a standard astronomical H-band optical
filter.

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The spectra were collected in interferogram form
using the interferometer and InSb detector of the spectro-
meter, and then converted to single beam spectrum
(intensity versus wavenumber) using the accompanying
5   Nicolet computer. All single beam spectra were
subsequently transferred to a Silicon Graphics Indigo
computer for data processing.

    Blood samples taken during the test were analyzed
for glucose using a Yellow Springs Instruments (YSI) model
10 2300 glucose analyzer.

    To process the spectra, the single beam spectra were
first converted to absorbance units by dividing each spec-
trum by a water reference spectrum and then computing the
negative logarithm. Only the portion of the spectrum
15 between 6500 and 5800 cm^{-1} was used in all subsequent
calculations.

    Spectra were then normalized using the signal
between 5955 and 5951 cm^{-1}, a wavenumber region that
contains no glucose information. The normalized spectra
20 were then digitally Fourier filtered to eliminate noise
and other spectral features that distract from glucose
information.

    The first step in the Fourier filtering process was
to perform a Fourier transform on the absorbance spectrum.
25 This resulted in a Fourier domain spectrum, which was then
multiplied by a Gaussian function defined by a mean
position of 0.037 f and a standard deviation width of
0.011 f. These parameters were selected to isolate the
glucose absorption. The filtered transformed spectrum
30 was then converted back to an absorbance spectrum by
applying an inverse Fourier transform.

    The spectra obtained in this way were analyzed using
the standard PLS regression procedure. This procedure
essentially goes into the data set and weights the

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different spectral frequencies by correlating spectral
variation with analyte concentration. The result is a
series of factors and regression coefficients which can be
applied to individual spectra to obtain a predicted value
5 of glucose concentration.

Fig. 10 shows the correlation between glucose
concentration as determined from the spectra and the
glucose level as determined from the blood samples taken.
The open symbols in the graph represent the data that were
10 used to generate the calibration model. The solid symbols
represent results from an independent set of spectra used
only for prediction purposes.

All of the results cluster around the unity line,
and the prediction points are within the general spread
15 and scatter of the calibration points. Thus, the ability
of the method of the invention to predict blood glucose
levels is confirmed. Moreover, since there is no
clustering of data according to individual, a global
calibration rather than individual calibrations may be
20 possible.

<u>EXAMPLE 3</u>

To evaluate the importance of the digital filtering
step in Example 2, the data taken in that experiment were
reevaluated with the digital filtering step omitted and a
25 separate set of PLS factors were developed. The resulting
plot of spectral glucose versus blood glucose measurements
is shown in Fig. 11. As is apparent, the data in this
case has a much poorer correlation. Thus, the importance
of an appropriate digital filtering technique is clear.

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EXAMPLE 4

To test the validity of the derived linear relation-
ship between concentration and interferogram peak
magnitude, a series of preliminary experiments were
5   performed using solution samples of benzene in CS_2. CS_2
has essentially zero absorbance in the region of the C-H
out-of-plane bending band of benzene at 1036 cm^{-1} that was
used as the targeted band for the analysis. The linear
approximation described above and a simple univariate
10 model were successful in relating the magnitude of the
filtered interferogram segments to concentration.

A second series of tests were run using samples of
glucose in phosphate buffer. The glucose C-H combination
band centered at 4400 cm^{-1} was used as the targeted band
15 for the analysis. This band is located on top of a broad
background absorbance of water spanning the 4000-5000 cm^{-1}
region, and thus presents a greater challenge since it
violates the assumption made in deriving the linear
relationship that only a single absorbing species would be
20 detected within the filter bandpass.

Calibration results made using the univariate linear
approximation exhibited substantial errors. Since the
information in the interferograms representing glucose and
water should still be additive, however, it was
25 hypothesized that a multivariate calibration model could
be used to correct for the interference due to water.
Partial least squares regression was used to build models
of the form

$$C = b_0 + b_1 x_1 + \ldots + b_n x_n$$

where C is glucose concentration and the n+1 b_i values are
30 regression coefficients determined from a multiple linear
regression analysis of the calibration data set. The
independent variables, x_i, are factor scores obtained from

- 25 -

the PLS analysis of the filtered interferogram segment.
The original independent variables used to obtain these
latent variables are the intensities of the individual
points in the filtered interferogram segment.

5   This approach was highly successful, yielding
excellent glucose calibration models. Models based on
three PLS factors were typically found to be optimal.
Standard errors of calibration and prediction for these
models after optimization of the bandpass filter wave-
10 length and width and the region of the interferogram being
analyzed ranged from 0.4 to 0.5 mM.

EXAMPLE 5

        Direct analysis was performed on interferogram data
for mixtures of glucose, bovine serum albumin (BSA) and
15 triacetin in pH 7.4, 0.1 M phosphate buffer. This syn-
thetic matrix provides many of the challenges of an actual
biological matrix, while offering control and reproduci-
bility. In particular, this matrix is challenging because
both BSA and triacetin have significant absorption in the
20 4000-5000 cm^{-1} range.

        As in the analysis of glucose in phosphate buffer
described in Example 4, multivariate calibration models
were necessary to account for the presence of multiple
absorbing species. In addition, utilization of multiple
25 filters centered on different parts of the spectral region
of interest was found to be effective.

        Specifically, filters of various widths which were
centered on the glucose band at 4400 cm^{-1}, the BSA band at
4600 cm^{-1}, and the triacetin band at 4440 cm^{-1} were studied.
30 Each filter was applied to the raw interferogram, produc-
ing one, two or three filtered interferograms depending on
the number of filters used. Segments of these filtered
interferograms were then used together in PLS analysis

- 26 -

Fig. 12 displays the data obtained from applying
three distinct filters to an interferogram obtained from a
glucose/BSA/triacetin sample. Segments of 1000 points
from each of the three filtered interferograms are
5   plotted, with the segments obtained by use of the glucose,
BSA and triacetin filters being plotted as points 1-1000,
1001-2000 and 2001-3000, respectively. Shorter segments
of each of these regions (denoted by the arrows in Fig.
12) were then investigated.
10   For cases using two and three filters, optimization
of eight and twelve experimental variables is required,
respectively. For each filter, the optimal bandpass
position and width must be found, as well as the optimal
interferogram segment location and length. This process
15 involves repetitive calculations on standard data sets and
evaluation of which factors provide the best ultimate
calibration set.

Fig. 13 plots the standard error of calibration
(solid line) and prediction (dashed line) versus the
20 number of PLS factors used to form the calibration model
in a two-filter analysis based on interferogram points
340-640 for the glucose filter and points 200-301 for the
BSA filter. The optimal model size in this case is 14
terms. This model exhibited an R^2 value of 98.45%, and
25 standard errors of calibration and prediction of 0.704 and
0.841 mM, respectively.

Fig. 14 is a correlation plot of glucose concentra-
tions estimated using this 14 term calibration model
versus the corresponding actual concentrations. Calibra-
30 tion samples are indicated by closed circles, while
prediction samples are indicated by open triangles.
Inspection of this data reveals that the prediction data
falls within the span of variation of the calibration data

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and that a strong correlation exists between estimated and
actual concentrations.

Examination of residuals from the model reveals
no relationship between residuals and either BSA or
5   triacetin concentration. There is some evidence of non-
linearity in the plot of residuals versus estimated
concentrations, however. This suggests that the presence
of signals from multiple absorbing species within the
filter bandpass has violated the assumptions of the linear
10   approximation equation set forth above.

EXAMPLE 6

The use of multivariate calibration models provides
the ability for the model itself to extract signals from
overlapping information in the interferogram. This raises
15   the question as to whether the digital filtering step is
necessary. To address this question, an optimization was
conducted to identify the best segment in the unfiltered
spectrum for use in building a multivariate calibration
model. The best model found was based on interferogram
20   points 100-450 and included 15 PLS factors. Figure 15 is
a correlation plot analogous to Figure 14 for this model.
As can be seen, the standard errors of calibration and
prediction have both increased by a factor of approxi-
mately three, and significantly greater scatter exists in
25   the data points. Thus, it appears that the filtering step
is essential to obtaining good calibration models from the
interferogram data.

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WE CLAIM:

2   1.   A method for non-invasive quantitative
3   detection of a physiological chemical in a test subject
4   comprising the steps of:
5   (a)   irradiating a portion of the test subject with
6   near-infrared radiation such that the radiation is
7   transmitted through or reflected from the test subject;
8   (b)   collecting data concerning the transmitted or
9   reflected infrared radiation using a detector;
10   (c)   digitally filtering the collected data to
11   isolate a portion of the data indicative of the
12   physiological chemical; and
13   (d)   determining the amount of physiological
14   chemical in the test subject by applying a defined
15   mathematical model to the digitally filtered data.

1   2.   A method according to claim 1, wherein the
2   digital filtering is performed by a Fourier filtering
3   process.

1   3.   A method according to claim 1, wherein the
2   collected data is in the form of an absorbance spectrum.

1   4.   A method according to claim 3, wherein the
2   spectrum is digitally filtered by performing a Fourier
3   transform on the absorbance spectrum; multiplying the
4   transformed spectrum by a Gaussian function; and applying
5   an inverse Fourier transformation to the multiplied
6   transformed spectrum.

1   5.   A method according to claim 4, wherein the
2   mathematical model applied to the digitally filtered
3   spectrum applies coefficients developed using a partial

- 29 -

4   least squares regression multivariate calibration
5   procedure.

1   6.   A method according to claim 5, wherein the
2   spectrum is digitally filtered by performing a Fourier
3   transform on the absorbance spectrum; multiplying the
4   transformed spectrum by a Gaussian function; and applying
5   an inverse Fourier transformation to the multiplied
6   transformed spectrum.

1   7.   A method according to claim 6, wherein the
2   spectrum is digitally filtered by performing a Fourier
3   transform on the absorbance spectrum; multiplying the
4   transformed spectrum by a Gaussian function; and applying
5   an inverse Fourier transformation to the multiplied
6   transformed spectrum.

1   8.   A method according to claim 7, wherein the
2   spectrum that is digitally filtered spans the region from
3   6500 to 5800 cm^{-1}.

1   9.   A method according to claim 8, wherein the
2   magnitude of the digitally filtered spectrum is compared
3   to the standard value using a series of regression
4   coefficients developed using a partial least squares
5   regression multivariate calibration procedure.

1   10.   A method according to claim 1, wherein the
2   collected data is in the form of a single beam spectrum.

1   11.   A method according to claim 1, wherein the
2   collected data is in the form of an interferogram.

- 30 -

1   12. A method according to claim 11, wherein only a
2   short segment of an entire interferogram is analyzed.

1   13. A method according to claims 11 or 12, wherein
2   the digital filtering step comprises the steps of
3   performing a fast Fourier transform on the interferogram
4   to obtain a transformed spectrum; multiplying the
5   transformed spectrum with a Gaussian function to select
6   for a peak diagnostic for the physiological chemical; and
7   applying an inverse Fourier transform to the multiplied,
8   transformed spectrum.

1   14. A method according to claim 13, wherein the
2   digital filtering step comprises the steps of performing a
3   fast Fourier transform on the interferogram to obtain a
4   transformed spectrum; multiplying the transformed spectrum
5   with a Gaussian function to select for a peak diagnostic
6   for the physiological chemical; and applying an inverse
7   Fourier transform to the multiplied, transformed spectrum.

1   15. A method according to claim 14, wherein the
2   Gaussian function is selected to isolate the glucose-
3   associated absorbance at 4400 cm^{-1}.

1   16. A method according to claim 1, wherein the
2   collected spectrum is an interferogram and the digital
3   filtering is performed directly on the interferogram to
4   select out waveforms within the interferogram having a
5   frequency equal to

$$\frac{frequency}{of\,signal} = 2 \times \frac{wavenumber}{of\,radiation} \times \frac{mirror}{velocity}$$

6   where the wavenumber has units of cm^{-1}, mirror velocity is
7   the velocity of mirror travel in the interferometer used

- 31 -

8   to generate the interferogram expressed in units of
9   cm/sec.

1   17.   A method according to any of claims 1-16,
2   wherein the chemical is glucose.

1   18.   A device for measurement of glucose
2   concentration in a sample comprising:
3   (a)   means for collecting data concerning near-
4   infrared radiation transmitted through or reflected from
5   the sample;
6   (b)   means for digitally filtering the collected
7   data to isolate a portion of data indicative of glucose;
8   (c)   means for applying a defined mathematical model
9   to the digitally filtered data, whereby the amount of
10 glucose in the test subject is determined; and
11   (d)   means for reporting the amount of glucose in
12 the sample.

1   19.   An apparatus for the non-invasive measurement
2   of one or more physiological chemicals in a test subject,
3   comprising,
4   (a)   means for irradiating a portion of the surface
5   of the test subject with near-infrared radiation such that
6   transmitted or reflected near-infrared radiation is
7   available for collection;
8   (b)   means for collecting data concerning the
9   transmitted or reflected near-infrared radiation;
10   (c)   means for digitally filtering the collected
11 data to isolate a portion of data indicative of the
12 physiological chemical;
13   (d)   means for applying a defined mathematical model
14 to the digitally filtered data, whereby the amount of
15 chemical in the test subject is determined; and

16   (e) means for reporting the amount of chemical in
17 the sample.
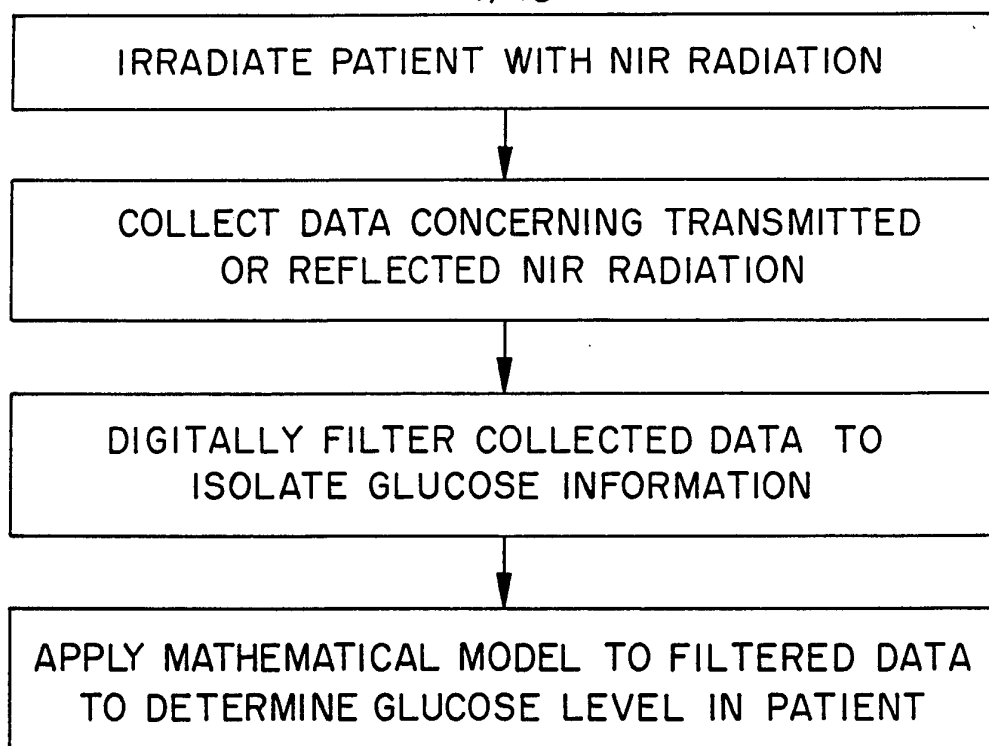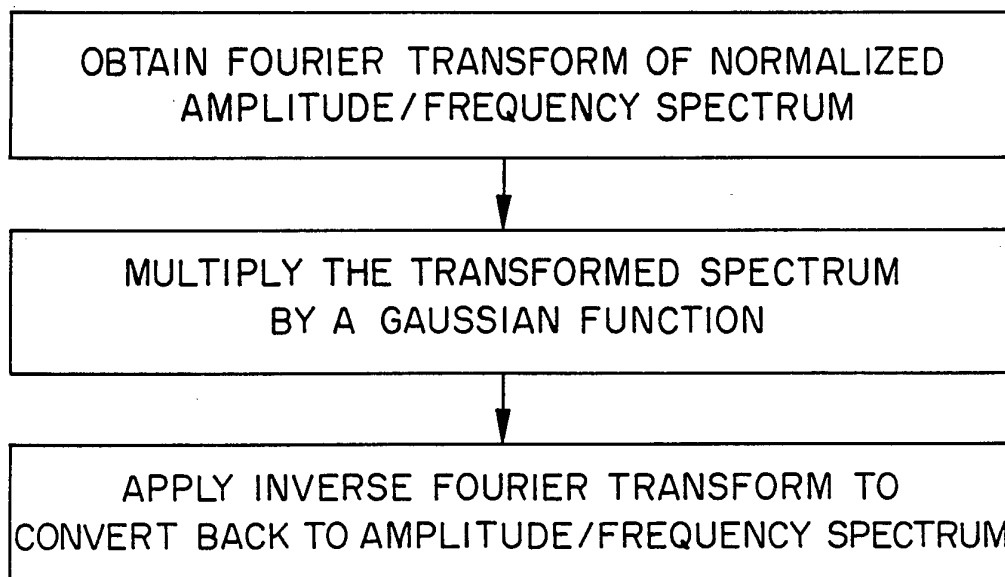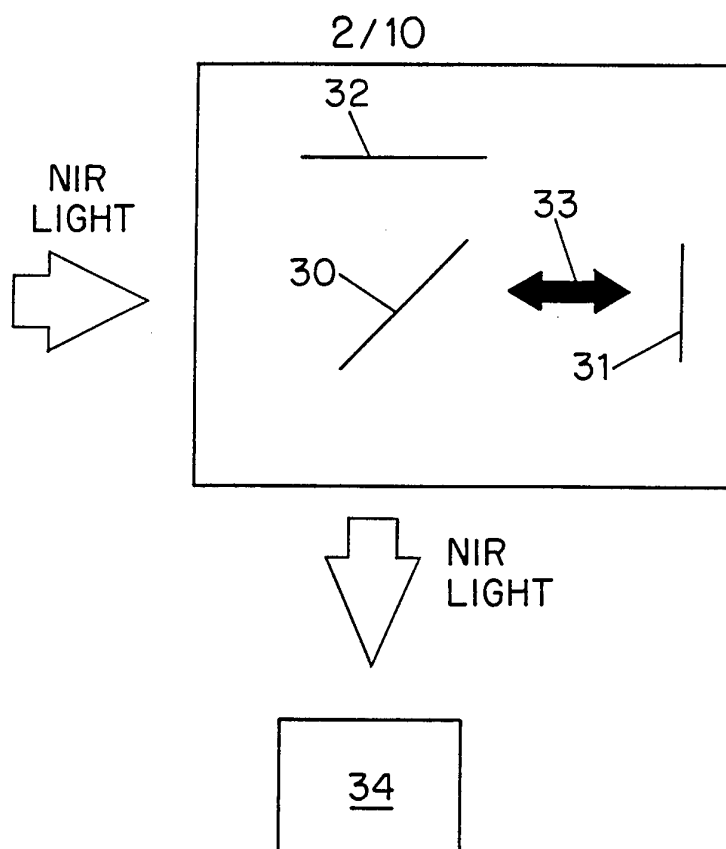
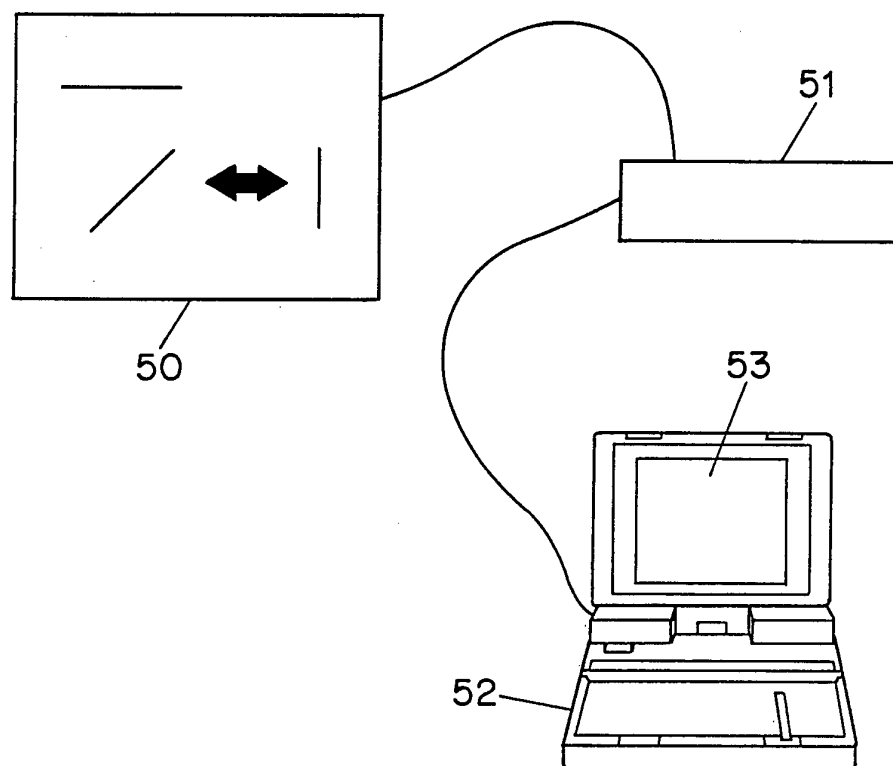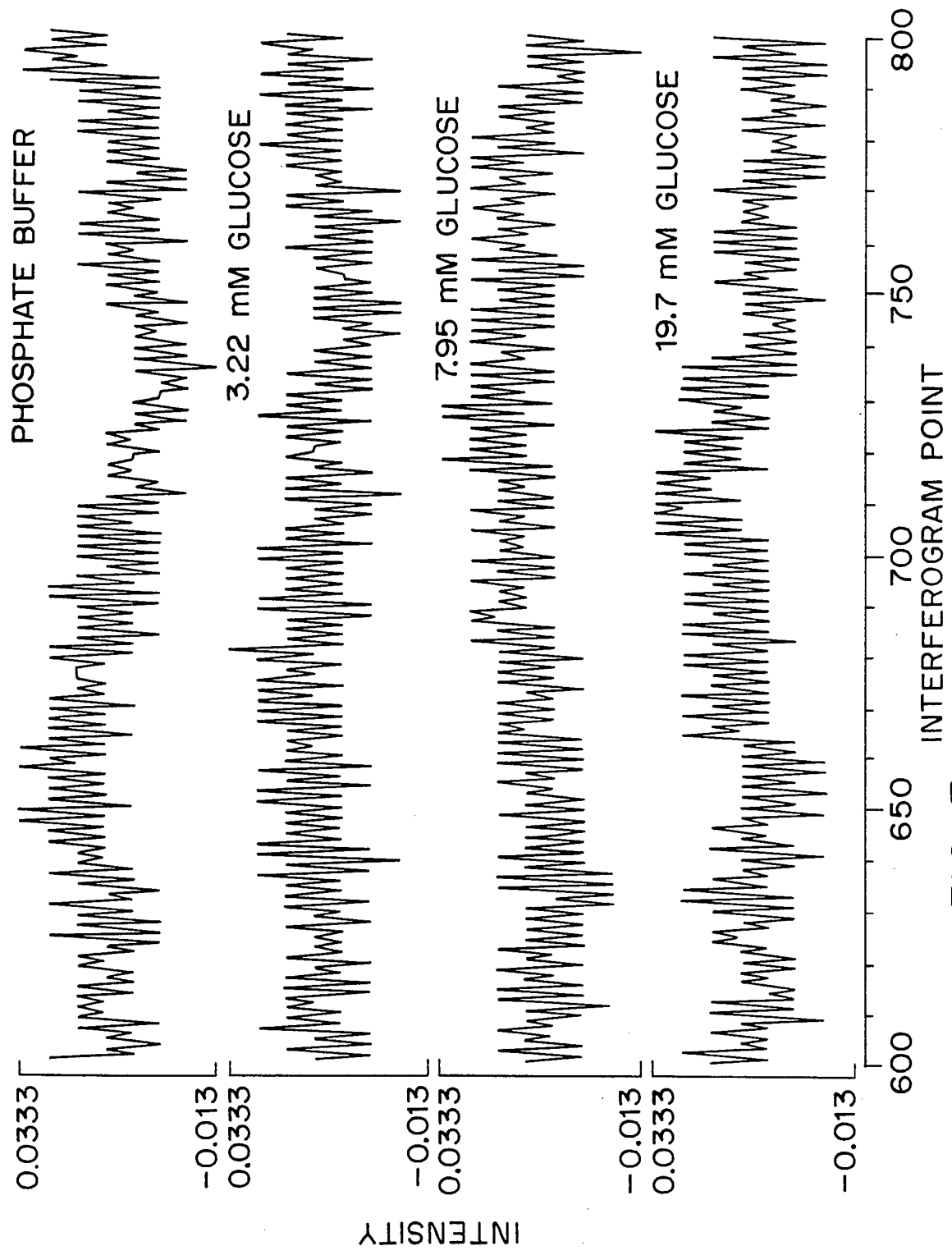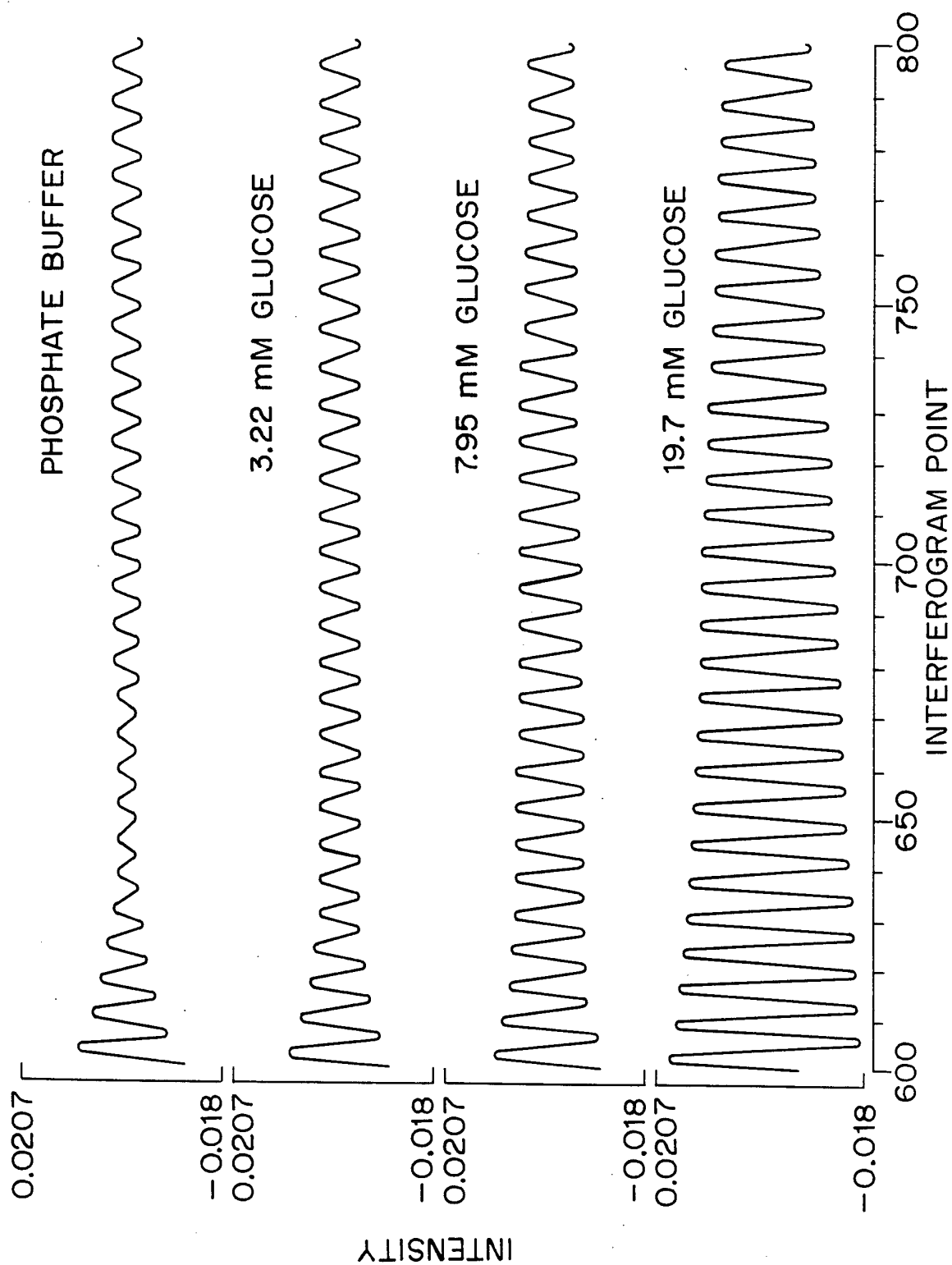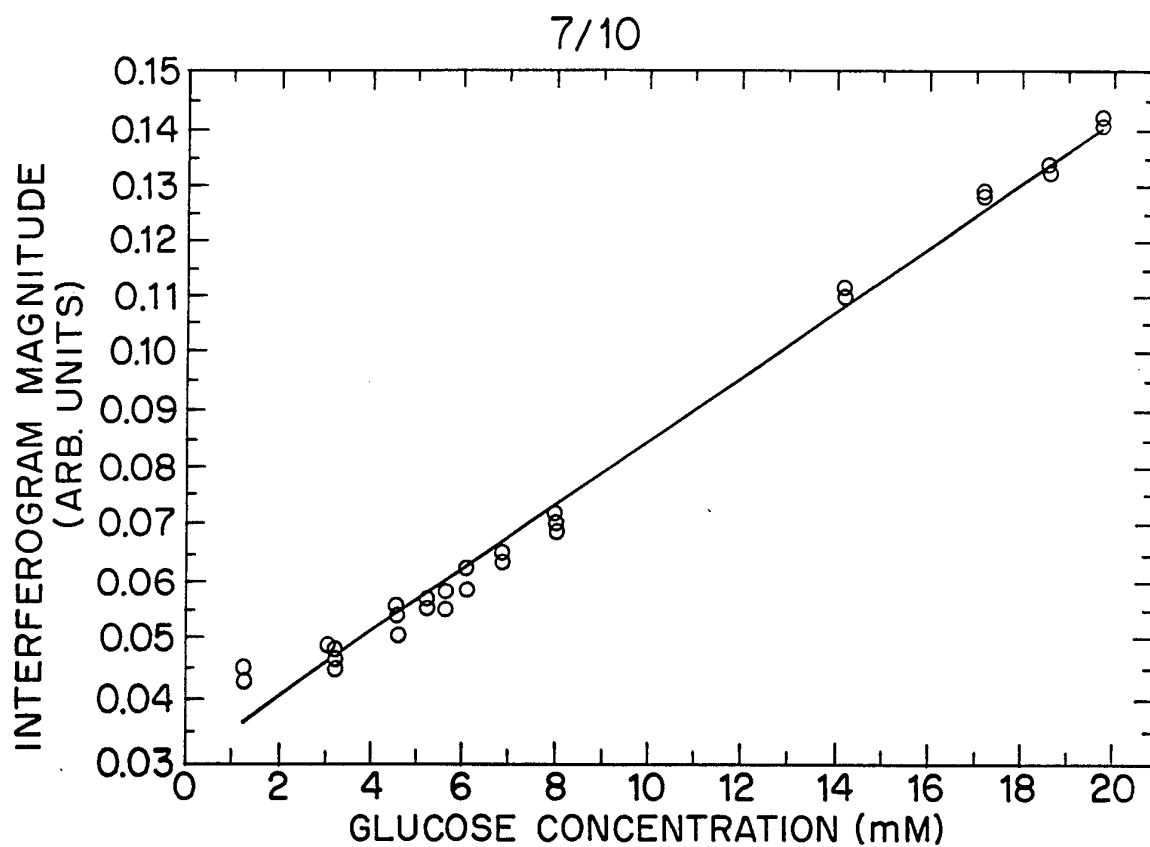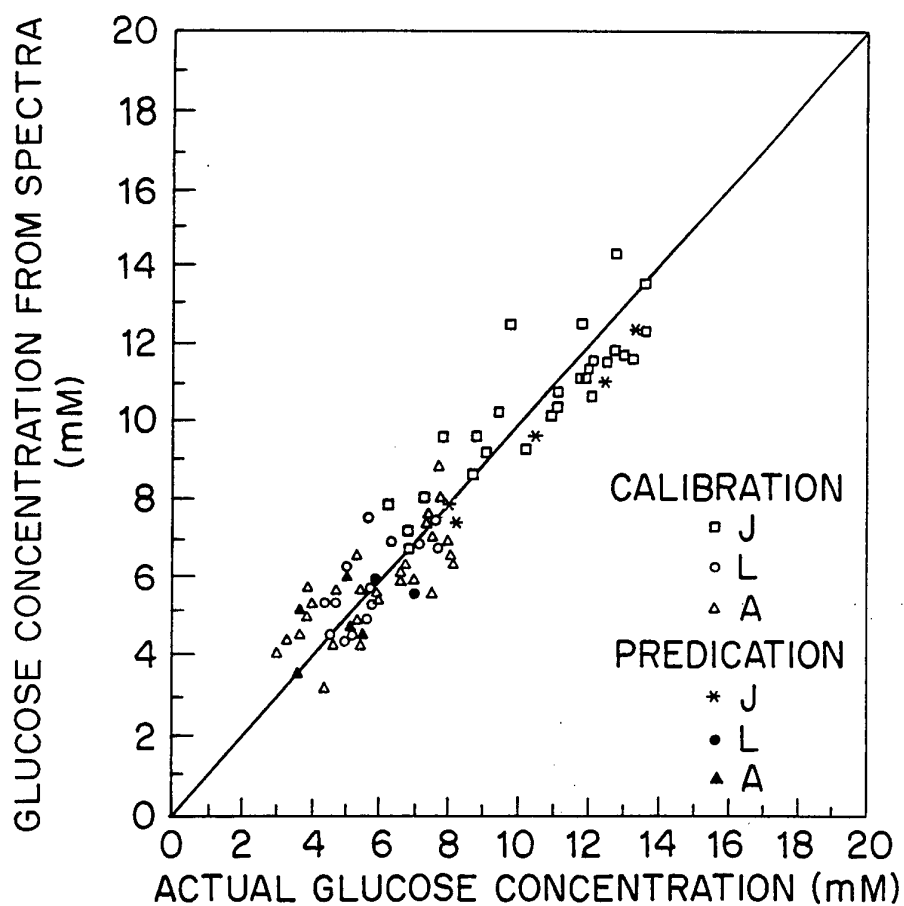IRRADIATE PATIENT WITH NIR RADIATION

COLLECT DATA CONCERNING TRANSMITTED
OR REFLECTED NIR RADIATION

DIGITALLY FILTER COLLECTED DATA TO
ISOLATE GLUCOSE INFORMATION

APPLY MATHEMATICAL MODEL TO FILTERED DATA
TO DETERMINE GLUCOSE LEVEL IN PATIENT

FIG. 1

OBTAIN FOURIER TRANSFORM OF NORMALIZED
AMPLITUDE/FREQUENCY SPECTRUM

MULTIPLY THE TRANSFORMED SPECTRUM
BY A GAUSSIAN FUNCTION

APPLY INVERSE FOURIER TRANSFORM TO
CONVERT BACK TO AMPLITUDE/FREQUENCY SPECTRUM

FIG. 2

2/10

NIR
LIGHT ⟹

32

33

30

31

NIR
LIGHT ⟱

34

FIG. 3

51

50

53

52

FIG. 5

3/10

FIG. 4

4/10

1.0000

FIG. 6A

D

65

FIG. 6B

FIG. 7

6/10

FIG. 8

FIG. 9

FIG. 10
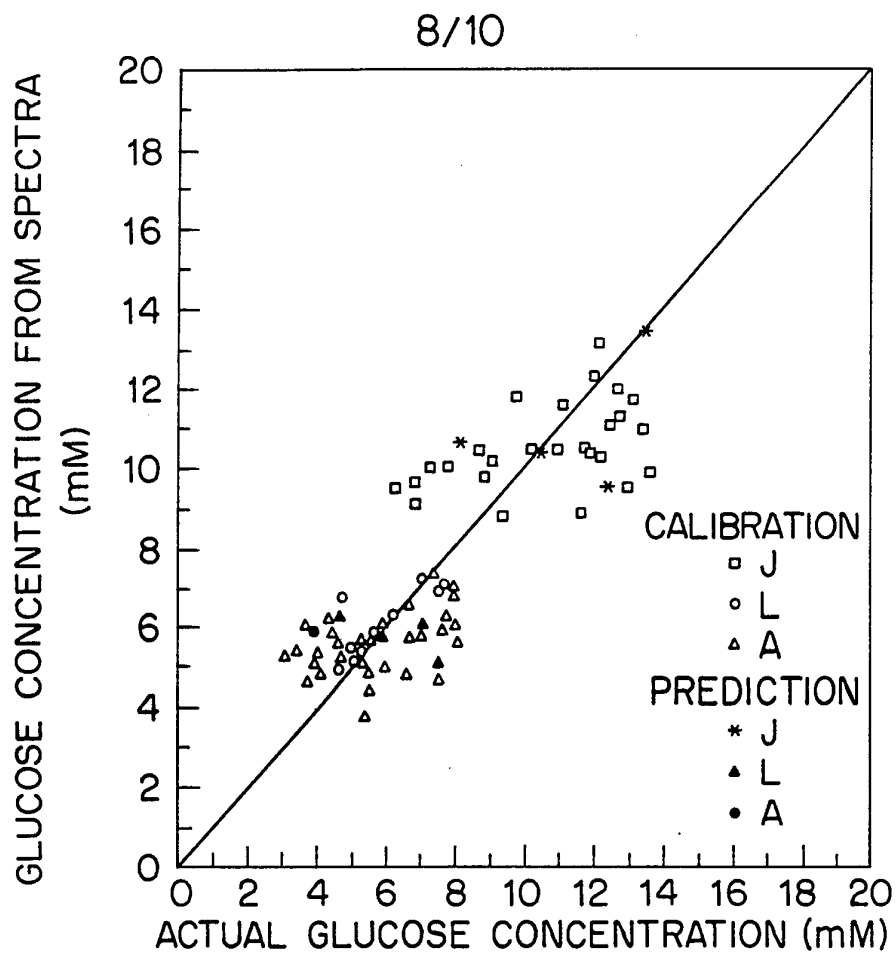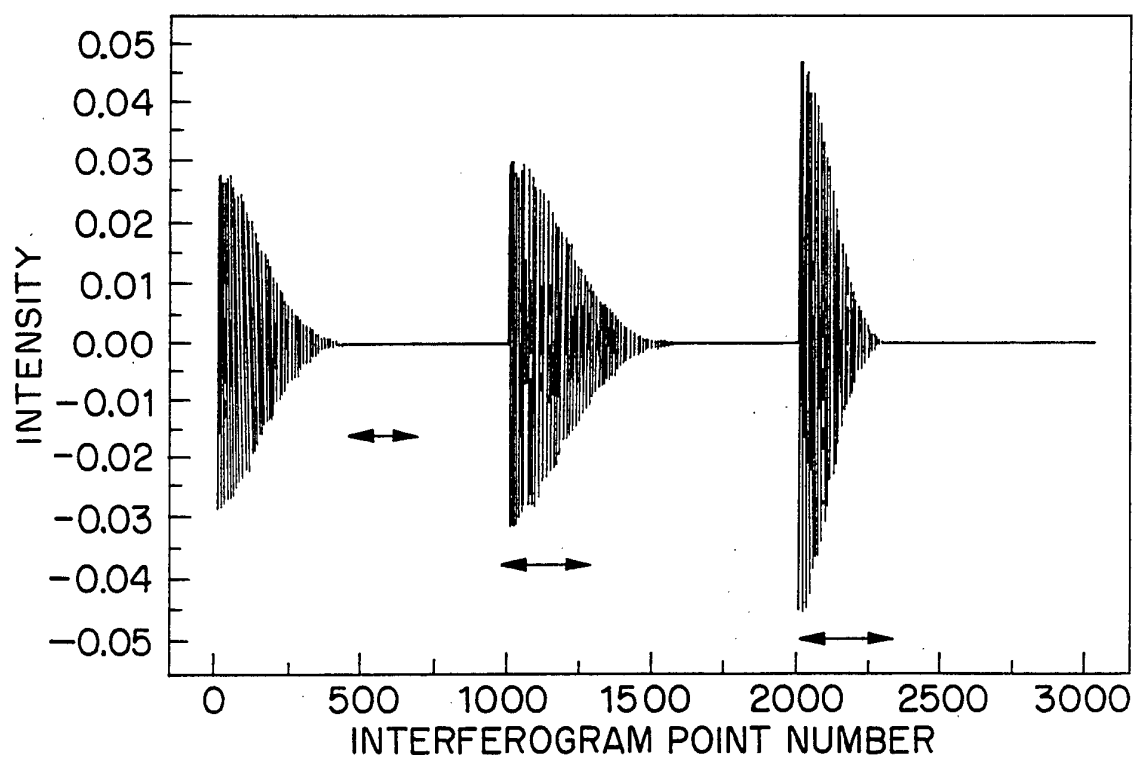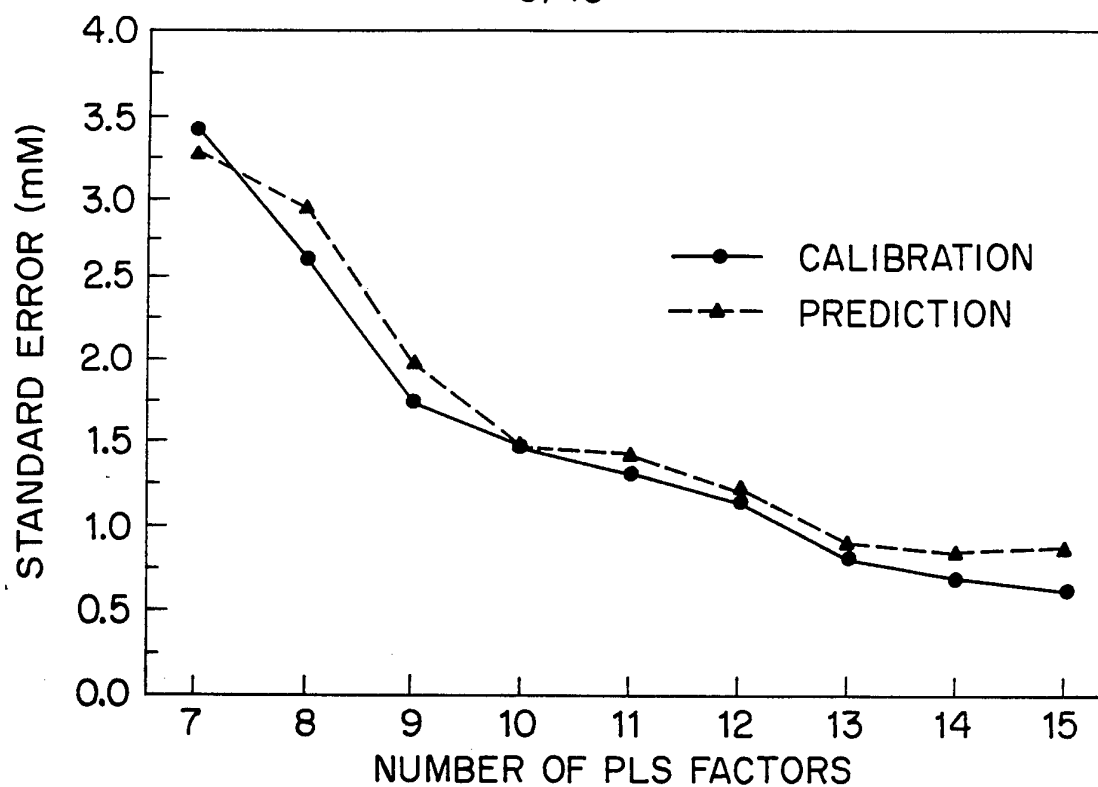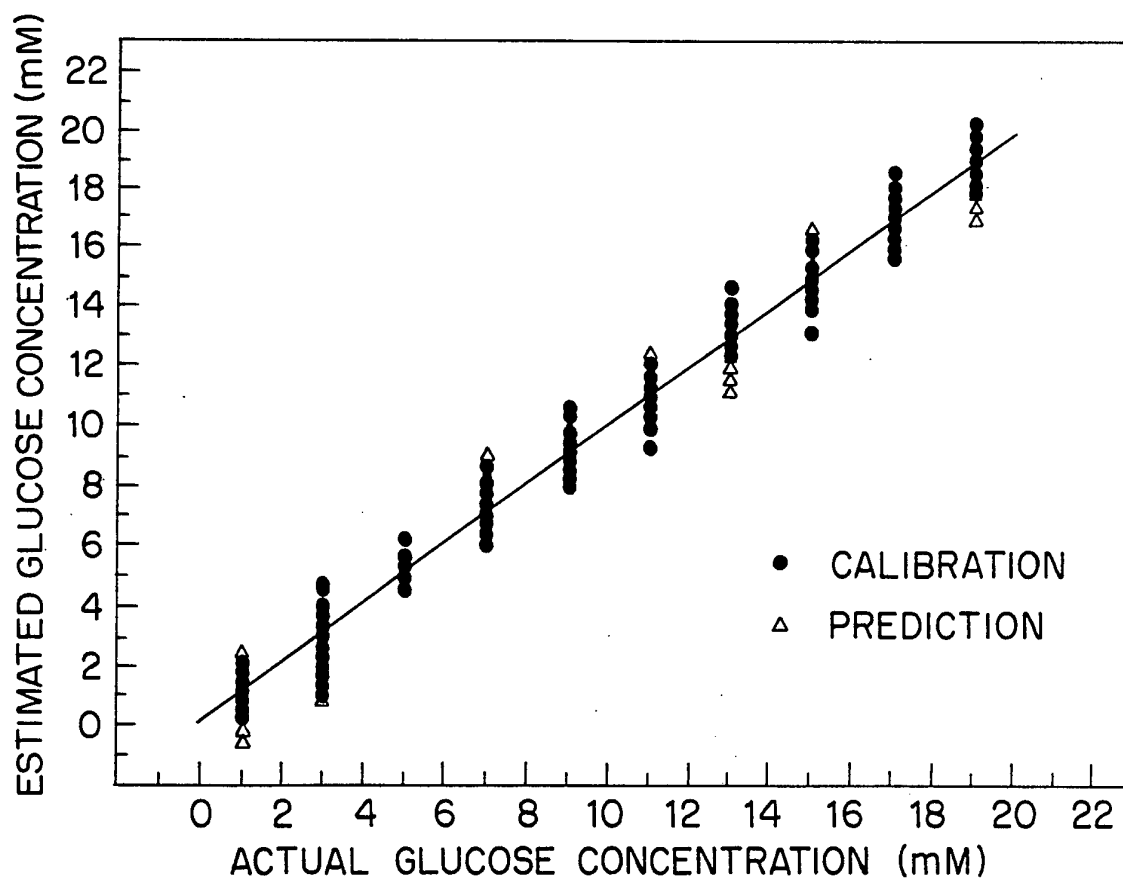
FIG. 11

FIG. 12

9/10

FIG. 13

FIG. 14
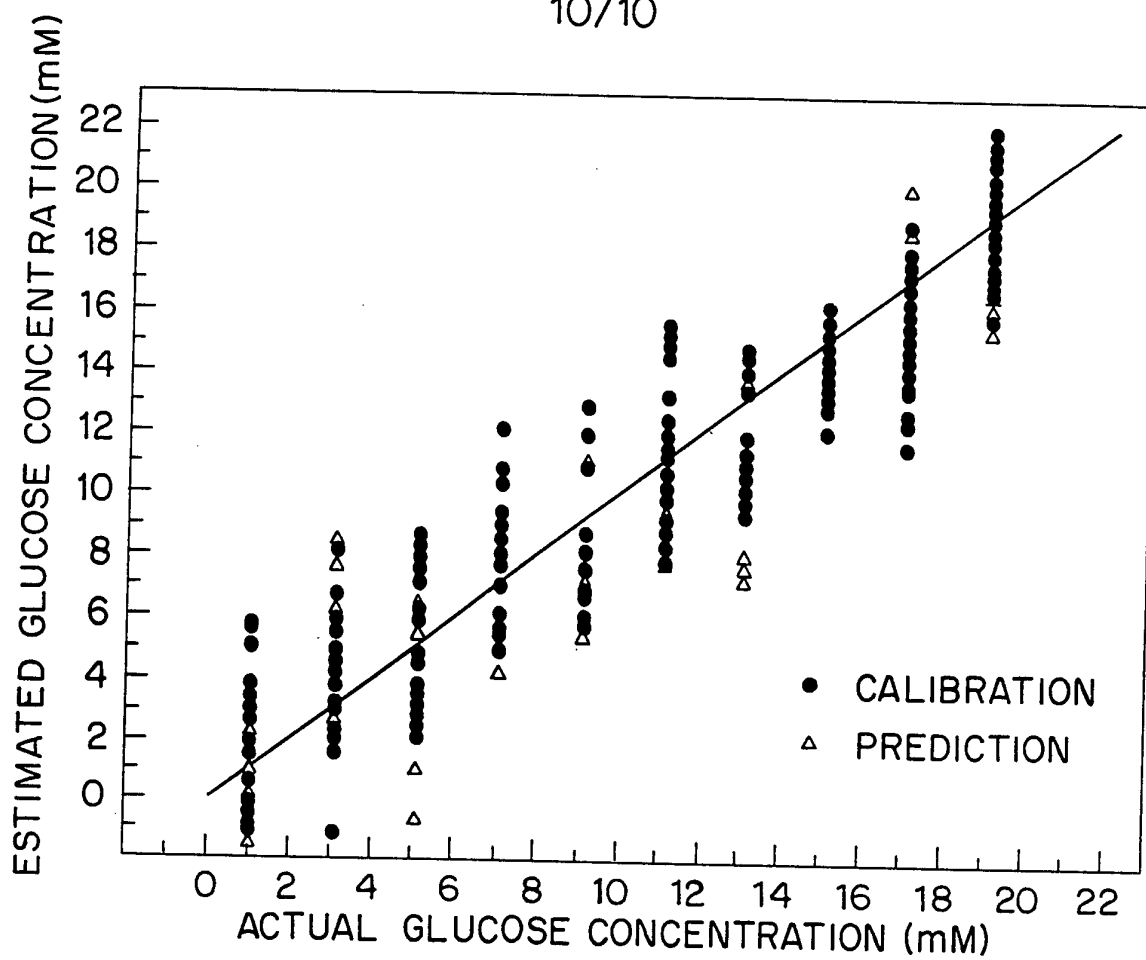
FIG. 15

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   G01N21/35   A61B5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6   G01N   A61B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US-A-5 086 229 (ROSENTHAL ROBERT D ET AL) 4 February 1992 cited in the application see column 2, line 51 - column 3, line 40; figure 2	1-3, 17-19
Y	EP-A-0 393 459 (MILCHWIRTSCHAFTLICHE FÖRDERUNGSGESELLSCHAFT MBH NIEDERSACHSEN) 24 October 1990 see column 1, line 53 - column 2, line 48; claims 1,3,4,6 see claim 1	1-3, 17-19
A	US-A-4 882 492 (SCHLAGER KENNETH J) 21 November 1989 see column 2, line 59 - column 3, line 36; figure 1	1,3, 17-19

-/--

[X] Further documents are listed in the continuation of box C.	[X] Patent family members are listed in annex.

* Special categories of cited documents :

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
7 June 1995	3 0. 06. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Krametz, E

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP-A-0 548 418 (DIASENSE INC) 30 June 1993 see column 3, line 11 - column 5, line 41; figure 1 -----	1,3, 17-19

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		AU-A-	8238791	23-01-92
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		JP-T-	5508336	25-11-93
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		US-A-	5068536	26-11-91
EP-A-393459	24-10-90	DE-A-	3922670	11-10-90
US-A-4882492	21-11-89	NONE		
EP-A-0548418	30-06-93	US-A-	5070874	10-12-91