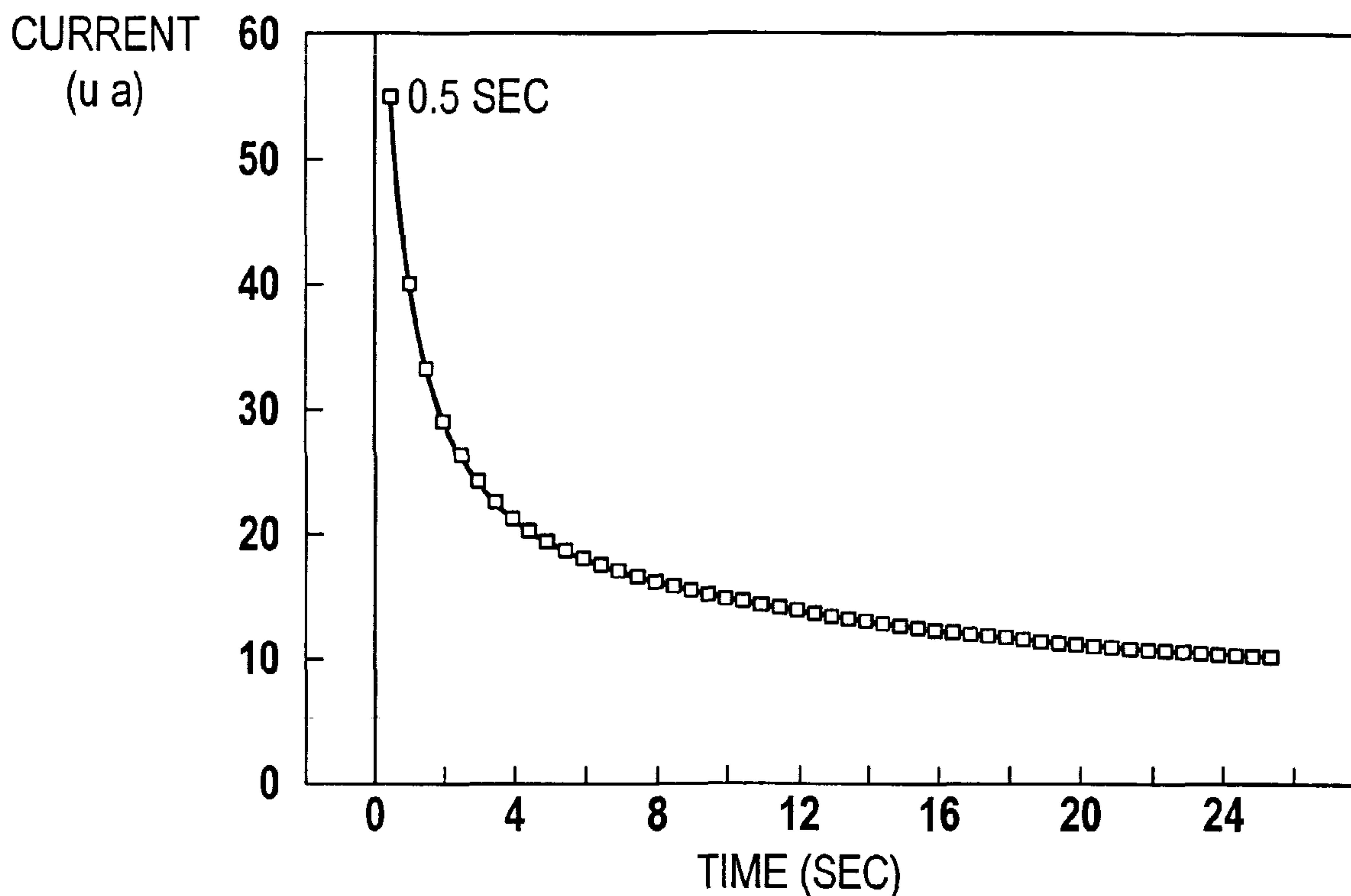




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 (71) Demandeur/Applicant:
F. HOFFMANN-LA ROCHE AG, CH
 (72) Inventeur/Inventor:
HELLER, ZINDEL HERBERT, US
 (74) Agent: OGILVY RENAULT

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 (54) Title: METHOD AND APPARATUS FOR THE DETERMINATION OF GLUCOSE



(57) Abrégé/Abstract:

A method and apparatus for performing a first measurement on a biological fluid or control, which first measurement varies with both the concentration of a first component and at least one of the presence and concentration of a second component. The method and apparatus perform a second measurement on the biological fluid or control, which second measurement varies primarily only with the at least one of the presence and concentration of the second component to develop an indication of the at least one of the presence and concentration of the second component. The first and second measurements may be made sequentially or simultaneously. The method and apparatus then remove an amount representative of the indicated presence or concentration of the second component from the concentration of the first component indicated by the first measurement.

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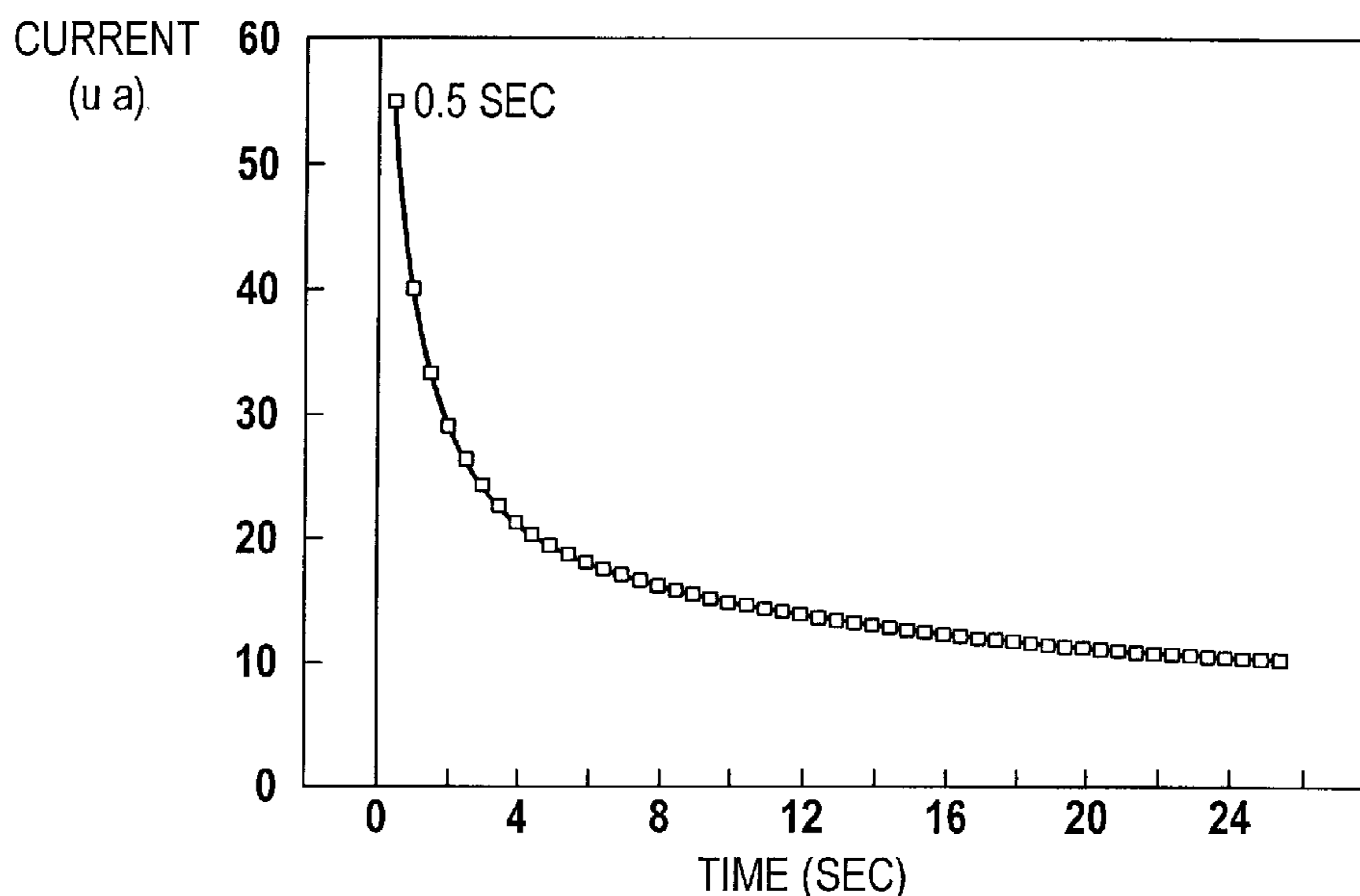
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- (71) Applicant (*for DE only*): **ROCHE DIAGNOSTICS GMBH** [DE/DE]; Sandhofer Strasse 116, 68305 Mannheim (DE).
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- (71) Applicant (*for all designated States except DE*): **F.HOFFMANN-LA ROCHE AG** [CH/CH]; Grenzacherstrasse 124, CH-4070 Basel (CH).
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(54) Title: METHOD AND APPARATUS FOR THE DETERMINATION OF GLUCOSE



(57) Abstract: A method and apparatus for performing a first measurement on a biological fluid or control, which first measurement varies with both the concentration of a first component and at least one of the presence and concentration of a second component. The method and apparatus perform a second measurement on the biological fluid or control, which second measurement varies primarily only with the at least one of the presence and concentration of the second component to develop an indication of the at least one of the presence and concentration of the second component. The first and second measurements may be made sequentially or simultaneously. The method and apparatus then remove an amount representative of the indicated presence or concentration of the second component from the concentration of the first component indicated by the first measurement.

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INSTRUMENT

Field of the Invention

This invention relates to methods and apparatus for the determination of the concentrations of biologically significant components of bodily fluids. It is disclosed in the context of methods and apparatus for the determination of glucose concentration in blood, a blood fraction or a control, but is believed to be useful in other applications as well.

Background of the Invention

15 Various methods and apparatus for the determination of the concentrations of medically significant components of body fluids are known. There are, for example, the methods and apparatus illustrated and described in the following listed references: U. S. Patents: 3,770,607; 3,838,033; 3,902,970; 3,925,183; 3,937,615; 4,005,002; 4,040,908; 4,086,631; 4,123,701; 4,127,448; 4,214,968; 20 4,217,196; 4,224,125; 4,225,410; 4,230,537; 4,260,680; 4,263,343; 4,265,250; 4,273,134; 4,301,412; 4,303,887; 4,366,033; 4,407,959; 4,413,628; 4,420,564; 4,431,004; 4,436,094; 4,440,175; 4,477,314; 4,477,575; 4,499,423; 4,517,291; 4,654,197; 4,671,288; 4,679,562; 4,682,602; 4,703,756; 4,711,245; 4,734,184; 4,750,496; 4,759,828; 4,789,804; 4,795,542; 4,805,624; 4,816,224; 4,820,399; 25 4,871,258; 4,897,162; 4,897,173; 4,919,770; 4,927,516; 4,935,106; 4,938,860; 4,940,945; 4,970,145; 4,975,647; 4,999,582; 4,999,632; 5,053,199; 5,011,290; 5,108,564; 5,128,015; 5,160,980; 5,232,668; 5,243,516; 5,246,858; 5,269,891; 5,284,770; 5,288,636; 5,312,762; 5,352,351; 5,366,609; 5,371,687; 5,379,214; 5,385,846; 5,395,504; 5,469,846; 5,508,171; 5,508,203; 5,509,410; 5,512,489; 30 5,522,255; 5,594,906; 5,686,659; 5,710,622; 5,789,664; 5,792,944; 5,832,921; 5,841,023; 5,942,102; and 5,997,817; WO98/35225; WO99/28736; and WO99/32881 and certain references cited in WO99/32881: German Patent Specification 3,228,542; European Patent Specifications: 206,218; 230,472; 241,309; 255,291; and, 471,986; and, Japanese Published Patent Applications JP 63-128,252 and 63-111,453.

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10 The disclosures of these references are hereby incorporated herein by reference. This listing is not intended as a representation that a complete search of all relevant prior art has been conducted, or that no better references than those listed exist. Nor should any such representation be inferred.

The clinically significant index of blood glucose, as used in the
15 diagnosis and management of diabetes, is its concentration in the serum, the clear fraction of whole blood after separation of red cells, white cells and plasma. The concentration is determined by any of a variety of chemical and electrochemical methods.

Typically, a predefined sample of serum is transferred from the blood
20 collection receptacle to a reaction vessel in which reagents are combined to produce a chemical reaction whose product is proportional to the total amount of glucose contained in the sample. The product of the chemical reaction can be quantified by gauging optical or electrochemical changes in the reaction mixture, represented as electrical signals or numerical values in digital format. Internal measurement units so
25 obtained, such as optical absorbency, microamperes, and so on, can be converted into reportable clinical units such as milligrams per deciliter or the like, by a blood glucose testing instrument calibration process performed as part of the overall clinical procedure. Internal instrument responses are measured on a series of serum-based reference standards, and the responses and corresponding concentration values are
30 plotted graphically to produce a calibration curve, or fitted computationally to a mathematical function representing concentration in terms of an instrument's response. Advances in computing since the development of many of the known clinical methods have permitted performance of complex data processing and calculation functions directly in even the smallest hand-held instruments.

35 In a serum-based assay system, the translated glucose concentration values are reported directly in units desired by the clinician. The excluded blood fractions do not figure either in the measurement or the translation process. This is not the case for a system in which the sample is in the form of whole blood. The

5 coexisting blood fractions may affect the reported values, either through physical or
chemical interference with the measurement process per se or due to their physical
displacement of serum in the sample volume. That is, because the wet chemistry
measures total glucose in the sample, the reported serum concentrations will vary as
the relative fraction of the serum component varies from sample to sample (usually
10 from individual to individual). In practice, the principle interference is from the red
and white blood cells.

For these and a variety of other reasons related to the manner in which
blood glucose monitors and test strips are distributed, the calibration procedure for
monitors is relatively complicated. Typically, calibration is performed in two steps.
15 In the first step, a large number of test strips in multiple whole blood monitors
measure a series of synthetic working standards having a range of predetermined
concentrations. The standards are quite stable, and are available in relatively large
quantities. The standards' values are used to construct (a) working relationship(s)
between the monitors' responses and the standards' concentrations. This step
20 provides a large number of determinations which are then pooled statistically for
increased precision over the monitor and test strip populations.

Then, in the second step, instrument response measurements are
performed on whole blood samples from a relatively smaller population of human
donors, which are paired with glucose values determined by the reference serum
25 methodology to adjust the working curve to yield true reportable values. This step
accounts on a statistical basis for the unique properties of human blood. Thus, human
donors provide, as they should, the ultimate basis for monitor results.

The decimal fraction of whole blood volume occupied by red cells is
known as hematocrit. Hematocrit correction on whole blood determinations would
30 not be necessary if hematocrit did not vary from person to person, because its effect
could be taken into account in the calibration process by introducing whole blood
samples into the instrument during calibration and relating their internally-measured
numbers to corresponding serum values determined during the reference phase of the
calibration process. However, hematocrit values can vary between about 0.2 for
35 individuals who suffer from anemia and about 0.6 for newborns. Since a glucose
determination is subject to about a one percent variation for each percent hematocrit
variation, the resulting uncertainty in indicated glucose concentration would be
clinically undesirable. Therefore, unless a glucose determination methodology is

5 employed which is hematocrit-independent, for closest agreement with laboratory
reference methods, individual determinations of glucose concentration must be
compensated or corrected for hematocrit. This applies both to the donors whose
blood is used during the calibration process and to the patients whose glucose
concentrations are of interest to the clinician.

10

Disclosure of the Invention

According to one aspect of the invention, a method is provided for
determining the concentration of a first, medically significant component of a
biological fluid or a control. The biological fluid or control includes a second
15 component which affects the determination of the concentration of the first
component. The method includes performing a first measurement on the biological
fluid or control, which first measurement varies with both the concentration of the
first component and at least one of the presence and concentration of the second
component. The method further includes performing a second measurement on the
20 biological fluid or control, which second measurement also varies with the at least
one of the presence and concentration of the second component, to develop an
indication of the at least one of the presence and concentration of the second
component. The method further includes removing an amount representative of the
indicated presence or concentration of the second component from the concentration
25 of the first component indicated by the first measurement.

Illustratively according to this aspect of the invention, the biological
fluid is blood or a blood fraction.

Further illustratively according to this aspect of the invention, the first
component is glucose.

30 Additionally illustratively according to this aspect of the invention, the
second component is blood cells.

Illustratively according to this aspect of the invention, the second
measurement is of a largely glucose-insensitive measure of hematocrit.

35 Additionally illustratively according to this aspect of the invention, the
first measurement is of a hematocrit-sensitive measure of glucose concentration.

Further illustratively according to this aspect of the invention, the
method includes contacting the biological fluid or control with a reactant before

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5 performing the first measurement in order to permit a reaction to take place between one of the first and second components and the reactant.

Illustratively according to this aspect of the invention, the first and second measurements exhibit an interdependence between the concentration of the first component and the concentration of the second component.

10 Further illustratively according to this aspect of the invention, performing a first measurement on the biological fluid or control, which first measurement varies with both the concentration of the first component and at least one of the presence and concentration of the second component, includes performing measurements of a time-varying function $i_1(t)$ having the general form

15
$$i_1(t) = M/\sqrt{t} + B$$

where t is time from initiating the measurement, M is the slope of a graph of the function and B is a value the function approaches as t becomes very large.

Additionally illustratively according to this aspect of the invention, performing a second measurement on the biological fluid or control, which second measurement varies primarily only with the at least one of the presence and concentration of the second component, to develop an indication of the at least one of the presence and concentration of the second component includes performing at least one measurement of a time-varying function $i_2(t)$, where t is time, $t <$ some arbitrarily established time. $i_1(t)$ and $i_2(t)$ may both exist in the circuit such that the aggregate current $i(t)$ at an output of the circuit is their sum, that is, $i(t) = i_1(t) + i_2(t)$ where the clocks which measure $i_1(t)$ and $i_2(t)$ are appropriately synchronized.

Illustratively according to this aspect of the invention, removing an amount representative of the indicated presence or concentration of the second component from the concentration of the first component indicated by the first measurement includes removing from the measurements of $i_1(t)$ the at least one measurement of $i_2(t)$.

According to another aspect of the invention, an apparatus is provided for determining the concentration of a first, medically significant component of a biological fluid or a control. The biological fluid or control includes a second component which affects the determination of the concentration of the first component. The apparatus includes a device for performing a first measurement on the biological fluid or control, which first measurement varies with both the

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5 concentration of the first component and at least one of the presence and
concentration of the second component. The device further performs a second
measurement on the biological fluid or control, which second measurement varies
primarily only with the at least one of the presence and concentration of the second
10 component to develop an indication of the at least one of the presence and
concentration of the second component. The device further removes an amount
representative of the indicated presence or concentration of the second component
from the concentration of the first component indicated by the first measurement.

Illustratively, apparatus according to this aspect of the invention
includes apparatus for determining the concentration of a first, medically significant
15 component of blood, a blood fraction or a control.

Further illustratively, apparatus according to this aspect of the
invention includes apparatus for performing the first measurement on the blood,
blood fraction or control, which first measurement varies with both the concentration
of glucose and the concentration of blood cells in the blood, blood fraction or control.

20 Additionally illustratively, apparatus according to this aspect of the
invention includes apparatus for performing the second measurement on the blood,
blood fraction or control, which second measurement varies primarily only with the
concentration of blood cells in the blood, blood fraction or control.

Illustratively, apparatus according to this aspect of the invention
25 includes apparatus for performing the second measurement of a largely glucose-
insensitive measure of hematocrit.

Additionally illustratively, apparatus according to this aspect of the
invention includes apparatus for performing the first measurement of a hematocrit-
sensitive measure of glucose concentration.

30 Further illustratively, apparatus according to this aspect of the
invention includes a reactant for contacting the biological fluid or control before
performing the first measurement in order to permit a reaction to take place between
one of the first and second components and the reactant.

Illustratively, apparatus according to this aspect of the invention
35 includes apparatus for performing first and second measurements which relate a non-
linear variation of the concentration of the first component with the concentration of
the second component.

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5 Further illustratively according to this aspect of the invention, the device for performing a first measurement on the biological fluid or control, which first measurement varies with both the concentration of the first component and at least one of the presence and concentration of the second component, includes a device for performing measurements of a time-varying function $i_1(t)$ having the
10 general form

$$i_1(t) = M/\sqrt{t} + B$$

where t is time from initiating the measurement, M is the slope of a graph of the function and B is a value the function approaches as t becomes very large.

15 Additionally illustratively according to this aspect of the invention, the device for performing a second measurement on the biological fluid or control, which second measurement varies primarily only with the at least one of the presence and concentration of the second component to develop an indication of the at least one of the presence and concentration of the second component, includes performing at least one measurement of a time-varying function $i_2(t)$, where t is some arbitrarily
20 established time.

Illustratively according to this aspect of the invention, the device for removing an amount representative of the indicated presence or concentration of the second component from the concentration of the first component indicated by the first measurement includes a device for removing from the measurements of $i_1(t)$ the at
25 least one measurement of $i_2(t)$.

Brief Description of the Drawings

The invention may best be understood by referring to the following detailed description and accompanying drawings which illustrate the invention. In the
30 drawings:

Fig. 1 illustrates a typical plot of current i versus time t for an instrument of the general type described;

Fig. 2 illustrates a typical plot of i versus $1/\sqrt{t}$;

35 Fig. 3 illustrates plots of M , the slope of Cottrell current $i(t)$ as a function of time t , versus glucose concentration at different hematocrits;

Fig. 4 illustrates an equivalent circuit model of an instrument and test strip of the general type described;

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5 Fig. 5 illustrates a schematic view of a glucose test strip of the general type described, with an applied sample, looking outward from the strip port on an instrument of the general type described;

Fig. 6 illustrates a waveform useful in understanding the invention;

Fig. 7 illustrates a waveform useful in understanding the invention;

10 Fig. 8 illustrates a waveform useful in understanding the invention;

Fig. 9 illustrates plots of D , the percentage initial current deficit, versus hematocrit at some arbitrarily short time after application of a step function voltage to a circuit of the type illustrated in Figs. 5-6; and,

15 Fig. 10 illustrates an equivalent circuit model of an instrument and test strip of the general type described.

Detailed Descriptions of Illustrative Embodiments

This application teaches an instrument and associated chemical and mathematical methods by which glucose and hematocrit are determined on a single
20 sample and a single test strip.

This application also teaches an instrument and methods by which a data stream from such an instrument's reaction sensor can be processed by internal computational apparatus in such a manner as to decompose the data into components separately representing hematocrit and glucose concentration which can then be
25 individually calibrated to report clinical values. In this regard, it is not necessary in diabetes management to report hematocrit. It is only necessary to free the measurement of glucose concentration from the effect of hematocrit. However, there are believed to be applications for a hand-held instrument which reports both values, such as, for example, an emergency room, a battlefield, and so on. In such
30 applications, numerical components separately representing glucose concentration and hematocrit can be individually calibrated and translated to report clinical values. And it is believed that the invention is useful in other systems besides glucose concentration and hematocrit. Using appropriate sensor technology, other analytes can be detected and their concentrations in a sample determined and reported.

35 The reaction dynamics may produce such an interaction between the two components that separating the results of the two may require solution of a pair of simultaneous equations by algebraic means or by regression analysis.

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5 Illustratively according to the invention, the two measurements proceed essentially simultaneously in a single passage of the sample through the instrument.

The need to account for the interference of other species with the determination of the concentration of a species of interest in biological fluids is well-
10 documented. Among the numerous proposals for accomplishing this objective are those described in WO99/32881 and certain references cited in WO99/32881. This application proposes another mechanism for accounting for the interference of other species in the determination of the concentration of a species of interest in biological fluids. Hematocrit is defined as the percentage of the blood's volume that is cells. In
15 instruments of the general types described in the above references, hematocrit interferes with the determination of glucose concentration of blood. This invention relates to the substantially simultaneous determination of hematocrit and indicated glucose concentration, which then permits indicated glucose concentration to be corrected for hematocrit to provide a more accurate indication of glucose
20 concentration.

Glucose measurements on whole blood are intrinsically sensitive to hematocrit, principally, it is believed, through the volume exclusion effect. That is, the greater the hematocrit, the less glucose is available for the glucose oxidase reaction. Hence, in order to better the accuracy of such instruments, it is necessary to
25 design the instruments and their glucose concentration determination algorithms around hematocrit, or to develop instruments and algorithms which measure hematocrit and compensate indicated glucose concentrations for the measured hematocrit, thus obtaining hematocrit-corrected glucose readings. An instrument 20 of the general type illustrated and described in U. S. Patents: 5,243,516; 5,288,636; 30 5,352,351; 5,385,846; and 5,508,171, for example, provides an incubation time, for example, ten seconds, for (a) reaction(s) to occur on a strip 22 of the general type illustrated and described in U. S. Patents: 5,243,516; 5,288,636; 5,352,351; 5,385,846; 5,508,171; and 5,997,817. Instrument 20 then applies across a pair of
35 electrodes 26, 28 of strip 22 a step function voltage 30, Fig. 6, to circulate a current of electrons collected from, and returned to, ions produced by the glucose-glucose oxidase reaction and associated reactions. Current readings are made at intervals of, for example, a half second. A first reading, taken a very short time, for example, .02

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5 sec., after application of the step function voltage 30, has previously been characterized by what were thought to be unpredictable artifacts. As a result, in prior art algorithms, such readings typically have not been used in the determination of glucose concentration.

10 The Cottrell equation predicts that current will decrease as the inverse of the square root of time after the application of the step function voltage 30. M is directly proportional to analyte concentration. Fig. 1 illustrates a typical plot of Cottrell current i versus time t . Fig. 2 illustrates typical plots of i versus $1/\sqrt{t}$. Five sets of these data are illustrated in Table 1.

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5

sample t	1	2	3	4	5	$1/\sqrt{t}$
0.0	144.23	133.68	144.34	137.70	149.78	
0.5	22.07	20.30	22.06	18.97	22.88	1.41
1.0	16.15	14.94	16.06	13.78	16.60	1.00
1.5	13.46	12.55	13.34	11.48	13.83	0.82
2.0	11.86	11.11	11.75	10.13	12.16	0.71
2.5	10.75	10.10	10.65	9.24	11.03	0.63
3.0	9.94	9.37	9.88	8.66	10.19	0.58
3.5	9.33	8.78	9.27	8.17	9.52	0.53
4.0	8.80	8.29	8.75	7.80	8.99	0.50
4.5	8.40	7.93	8.36	7.49	8.56	0.47
5.0	8.03	7.62	7.99	7.21	8.19	0.45
5.5	7.69	7.34	7.68	7.00	7.85	0.43
6.0	7.44	7.07	7.44	6.78	7.58	0.41
6.5	7.20	6.85	7.16	6.60	7.33	0.39
7.0	6.98	6.64	6.98	6.45	7.14	0.38
7.5	6.80	6.49	6.76	6.26	6.93	0.37
8.0	6.61	6.30	6.61	6.14	6.74	0.35
8.5	6.43	6.18	6.46	6.05	6.59	0.34
9.0	6.27	6.03	6.31	5.93	6.44	0.33
9.5	6.18	5.90	6.18	5.83	6.31	0.32

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10.0	6.02	5.81	6.06	5.74	6.16	0.32
10.5	5.90	5.69	5.91	5.62	6.07	0.31
11.0	5.81	5.60	5.82	5.56	5.94	0.30
11.5	5.72	5.51	5.69	5.46	5.85	0.29

5

Table 1

Unless otherwise specified, in the tables and discussions that follow, current is measured in microamperes (μA), time in seconds (sec), and glucose concentrations in milligrams/deciliter (mg/dl). As will be appreciated, these plots are quite linear, and can be characterized by the equation:

10

$$i(t) = M/\sqrt{t} + B \quad (1)$$

where M is the slope of the Cottrell current curve and B is the asymptotic current, at $t = \infty$.

15

In the data studied, the B parameter was found to yield no independent correlation with glucose concentration, and considerably more variation; hence it was dropped from further consideration in the analyses.

20

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Although an assay for glucose could be based solely on the M parameter, a series of measurements with specially-prepared whole blood samples showed that for greater accuracy, hematocrit effects on indicated glucose concentration must be taken into account. Fig. 3 illustrates plots of M versus glucose concentration at different hematocrits for an illustrative set of data. The constant, or iso-, hematocrit contours form a manifold of separated, nearly straight lines that converge at a point near zero glucose concentration. The lines' slopes indicate relatively high sensitivity to variation in glucose concentration. However, there definitely is also hematocrit dependence. Given a measured M , the sought-after glucose concentration might fall on any line in the manifold, depending upon the hematocrit of the sample.

30

In order to learn whether a single measurement process that would be compatible with an instrument primarily designed to measure the Cottrell current could be configured to concurrently provide an index of hematocrit, the electrical properties of the strip and measuring circuit were studied in further detail. Fig. 4 illustrates an equivalent circuit model that models with a few conventional circuit

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5 elements the electrical performance of instrument 20 and strip 22. The circuit
 illustrated in Fig. 4 includes a resistor 32 that represents the ionic current flowing in
 the bulk of the reaction volume, as distinct from the Cottrell current source 34 that
 represents the electrochemical charge transfer and exhibits the $1/\sqrt{t}$ variability noted
 in the above equation. The sum of these two currents is provided to a current-to-
 10 voltage (i-v) converter 36, the output of which is a voltage across a feedback resistor
 38. The output voltage of converter 36 is then sampled and analog-to-digital (A/D)
 converted for further processing by other circuitry (not shown) in the instrument 20.

In practice, for $t > 1$ sec., the Cottrell current generator 34 and resistors
 32 and 44 accurately represent the equivalent circuit. However, for a rapidly
 15 increasing voltage, such as the leading edge of a step function, the strip capacitance
 40 acts as a transitory short circuit and Cottrell current cannot build up until the
 voltage across capacitance 40 is charged up, through resistor 44, to the step height.
 For the circuit parameters at hand, this time is less than a tenth of a second.
 Accordingly, for very short times ($t < 1$ sec.), the capacitance 40 associated with strip
 20 22 and sample 42 must also be included in the model, in parallel with the resistor 32
 and the Cottrell current source 34. Following completion of the charge-transfer
 process, resistors 32 and 44 account for the residual current represented by the B
 parameter.

Capacitance 40 can be appreciated by referring to Fig. 5, a schematic
 25 view of strip 22 with an applied sample 42, looking outward from the strip 22 port on
 instrument 20, that is, along the strip 22 electrodes 26, 28 toward the applied sample
 42. The electrodes 26, 28 of strip 22 form a pair of parallel plates 26, 28 having areas
 A, separated by a distance d. An illustratively somewhat hemispherical space above
 strip 22 and between the electrodes 26, 28 is filled with the sample 42 including
 30 whatever reagent(s), etc., are dissolved, suspended, or otherwise present, with or in
 the sample 42. Since blood is a composite material including two main components,
 plasma (the main liquid component of blood) and red blood cells (the main cellular, or
 solid, component of blood), the capacitance 40 should vary with hematocrit, to the
 extent that the dielectric properties of plasma and red blood cells differ. This
 35 expectation forms the basis of Fricke's equation:

$$(K-K_s) / (K+S \times K_s) = H \times (K_c-K_s) / (K_c+S \times K_s) \quad (2)$$

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5 wherein: K = the composite dielectric constant of the sample 42; K_s = the dielectric constant of the serum component; K_c = the dielectric constant of the red blood cells; H = the hematocrit; and S = a so-called "shape factor" related to the architecture of the strip 22, illustratively about 1.8. The dielectric constant of whole blood is directly proportional to its hematocrit. Because the capacitance 40 of a strip 22 is directly
10 proportional to the dielectric constant of its contents, equation (2) suggests that a determination of strip capacitance 40 would provide independent knowledge of the hematocrit of the sample.

Capacitance 40 can be quantified in a number of ways. For this application, the method involves utilizing the same hardware as an instrument
15 designed to measure Cottrell current exclusively, while revising the associated software. Referring particularly to Fig. 7, if instrument 20 applies a step function voltage 30 across the strip 22 electrodes 26, 28, that voltage step 30 must charge the capacitance 40 of strip 22 and sample 42. As capacitance 40 is charged, the voltage across the strip electrodes 26, 28 asymptotically approaches a steady state value. The
20 charging current spike has an initial value equal to the amplitude of the voltage step 30 divided by the internal resistance 44 of instrument 20, plus the leakage current of strip 22. Then, as the charge stored in capacitance 40 increases further, the current through strip 22 settles to the $1/\sqrt{t}$ -related Cottrell current as described by the above equation. Thus, the current at any instant is the sum of these two, and any deficit
25 between the actual current and the Cottrell current can be attributed to capacitance 40. Fig. 7 illustrates such a sum current trace. Fig. 8 illustrates the Cottrell current component calculated from $t = 0.5$ second onward. D is the percent deficit between the sum current and the Cottrell current extrapolated from $t = 0.5$ sec. backward toward $t = 0$, and includes the current flow immediately after voltage is applied at $t =$,
30 for example, 0.02 sec. D as a percentage of the total current is used because it is related to hematocrit, which is a percentage.

An investigation of the initial current spike was then undertaken. Fig. 9 illustrates plots of the percentage initial current deficit, hereinafter D , versus hematocrit at some arbitrarily short time after application of the step function voltage
35 30, again, illustratively $t = 0.02$ sec. These plots are based upon the same set of data as the plots illustrated in Fig. 3. Each plot provides information on both glucose

- 5 concentration and hematocrit. Table 2 illustrates how D is calculated for particular sets of data.

data group	1	2	3	4	5	mean of data groups	standard deviation of data groups	coefficients of variation of data groups
Cottrell current slope M at time t	14.89	13.39	14.82	11.93	15.33	14.07	1.25	8.91
Cottrell curve intercept B	1.27	1.56	1.28	1.88	1.30	1.46	0.24	16.30
measured i(t) - B	142.96	132.12	143.06	135.82	148.48	140.49	5.80	4.13
Cottrell component	105.29	94.71	104.79	84.36	108.39	99.51	8.86	8.91
Deficit D	37.67	37.41	38.27	51.46	40.09	40.98	5.32	12.98
D/(i(t) - B) × 100%	26.35	28.32	26.75	37.89	27.00	29.26	4.36	14.91

Table 2

5 Table 3 contains calculated values for M and D for each of the fifteen data points (three different glucose concentrations times five different hematocrits) in one run.

glucose concentration	40	40	120	120	450	450
hematocrit	D	M	D	M	D	M
20	17.1	16.0	5.4	32.9	-36.4	101.2
35	24.3	14.4	14.2	27.0	-15.3	78.4
45	29.8	14.6	22.7	23.3	-3.9	71.9
55	36.4	10.8	35.6	16.6	12.9	48.5
70	39.4	9.4	40.1	14.4	22.1	37.0

Table 3

10 The relationships among M, glucose concentration and hematocrit can be developed from the contents of Table 3. To accomplish this, M in Table 3 is regressed on a suitable set of variables. First, the shapes of the curves in Fig. 3 suggest a relationship of the form

$$M = m_0 + m_1 \times h + m_2 \times g + m_3 \times h \times g \quad (3)$$

15 where $h = (\text{hematocrit} - 0.45)$ and $g = (\text{glucose concentration} - 120)$. Similarly, study of the curves of Fig. 9 suggest a relationship for D having the form

$$D = d_0 + d_1 \times h + d_2 \times g + d_3 \times h \times g. \quad (4)$$

20 Regression analyses on these two equations yield the following values for the constants: $m_0 = 23.33$; $m_1 = -37.83$; $m_2 = 0.1333$; $m_3 = -0.2843$; $d_0 = 23.14$; $d_1 = 66.54$; $d_2 = -0.0823$; and, $d_3 = 0.1669$. These regression parameters m_0, m_1, m_2, m_3 and d_0, d_1, d_2, d_3 represent this database. That is, given a new sample taken from the same sample universe, and of known h and g values, the corresponding M and D values, measured on the same reagent lot and monitor instruments, could be predicted using these parameters in equations (3) and (4). Alternatively, as would be the case in

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5 practice, the equations (3) and (4) can be inverted, numerically or algebraically, to yield the glucose and hematocrit values for a pair of M, D values measured on a sample whose glucose and hematocrit values were unknown.

This analysis of strip 22 with applied sample 42 was developed based on the step function response of the measurement strip to isolate the capacitance 40. Whereas $i_2(t)$, the charging of the relatively small strip 22 capacitance 40 through the relatively low output resistance 44 is essentially complete in the first second, $i_1(t)$, the Cottrell current, persists for several seconds, which permits determination of the Cottrell current trace and extrapolation back in time to extract a single value of $i_2(t)$ from the sum current $i(t)$. Other means known to circuit designers could be employed.

For example, the circuit illustrated in Fig. 10 provides an alternative technique for analyzing the behavior of the strip 22/applied sample 42 that is compatible with the measurement parts of instrument 20 and strip 22, but circuit and software are revised to give a longer measurement interval for the capacitance. In the circuit realization illustrated in Fig. 10, during reading of the current between the electrodes 26, 28 of strip 22, a programmed voltage source 48 and internal resistance 44 are coupled in series between the instrument 20 ground, or common, terminal and the anode of a blocking diode 50. The cathode of blocking diode 50 is coupled through the parallel combination of Cottrell current generator 34, asymptotic current resistor 32 and capacitor 40 to the inverting, or -, input terminal of a differential amplifier 36. The - input terminal of amplifier 36 is coupled through a parallel RC feedback circuit including a feedback resistor 38 and a feedback capacitor 52, to its output terminal. The measurement of the current through the strip 22 proceeds as described above, and the output from amplifier 36 is sampled and A/D converted for further processing by the instrument 20.

Then, after the measurement cycle of the current through the strip 22, the strip 22 capacitance 40 is discharged to determine the charge accumulated on it during the current measurement phase. The programmed voltage source 48 is stilled, and a Field Effect Transistor (FET) switch 54 is turned on. When this condition occurs, ground is coupled through a bypass diode 56 to the parallel combination of the Cottrell current generator 34, resistor 32 and capacitor 40 and through the FET switch 54 to the - input terminal of an amplifier 60. The - input terminal of amplifier 60 is

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5 coupled through the parallel combination of a feedback resistor 62 and a feedback capacitor 64 to its output terminal. The - input terminal of amplifier 60 is also coupled through the feedback resistor 62/feedback capacitor 64 parallel combination to its output. The output from amplifier 60 is sampled and A/D converted for further processing by the instrument 20.

10 The programmed voltage source 48, internal resistance 44, blocking diode 50, bypass diode 56, amplifiers 36, 60, and their associated feedback resistors 38, 62, and capacitors 52, 64, and FET switch 54 are, of course, within the instrument 20. Strip 22 is modeled by the Cottrell current source 34, resistor 32 and capacitor 40 coupled in parallel between the cathode of blocking diode 50 and the cathode of
15 bypass diode 56. During current measurement, FET switch 54 is off, isolating the strip 22 circuit model from the inverting (-) input terminal of amplifier 60. When current measurement is complete, the FET switch 54 is turned on, and the strip 22 capacitance 40 is measured through instrument 20's amplifier 60, the output of which can be A/D converted by instrument 20 and used to calculate capacitance 40.

20 Both embodiments discussed above obtain hematocrit-free glucose determinations or simultaneous determinations of both hematocrit and glucose, making use of their relationships to circuit elements in the equivalent circuit of Fig. 4. This is accomplished by measuring some feature of the output current of the circuit subject to step function input voltage 38 of a waveform that rapidly rises to a steady
25 state value which is maintained for the duration of the measurement interval. The current amplitude over time, normalized to that of the step, is known as the indicial admittance. See, for example, E. Weber, Linear Transient Analysis, John Wiley and Sons, N. Y., 1954. Such indicial admittance completely characterizes the electrical properties of the circuit, and can be transformed mathematically to resolve values for
30 the individual circuit elements.

This resolution method can bypass such a laborious transformation by temporal separation of the two current contributors. Whereas $i_2(t)$, the charging of the relatively small strip 22 capacitance 40 through the relatively low output resistance 44 is essentially complete in the first second, $i_1(t)$, the Cottrell current, persists for
35 several seconds, which permits determination of the Cottrell current trace and extrapolation back in time to extract a single value of $i_2(t)$ from the sum current $i(t)$.

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5 Because of the parallel nature of the circuit, $i_2(t)$ is the indicial admittance of the strip and sample, a single point of which has sufficed to illustrate the principle of identifying separate analytes with separate circuit elements.

Linear Transient Analysis, referenced above, teaches at page 173 that a circuit's properties can be equivalently characterized by its impedance function, the ratio of amplitude of an applied sinusoidal voltage $V(\omega)$ to the complex circuit current $I(\omega)$, V/I as a function of frequency ω . The temporal and frequency representations are related by a set of integral equations, also taught at Linear Transient Analysis, page 73, that are readily reduced to transform between them. Thus, the measurement could be made by using a constant-amplitude, swept frequency, sinusoidal voltage generator, and recording the amplitude and phase of the output sinusoidal current, the two methods being electrically entirely equivalent. As above, various indices of the real and imaginary components of the impedance function can be extracted which, when regressed on assay values in a calibration reference set, will yield relations for the assay values of patient samples.

10

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5 Claims

1. A method for determining the concentration of a first, medically significant component of a biological fluid or a control, the biological fluid or control including a second component which affects the determination of the concentration of the first component, the method including performing a first
10 measurement on the biological fluid or control which first measurement varies with both the concentration of the first component and at least one of the presence and concentration of the second component, performing a second measurement on the biological fluid or control which second measurement also varies with the at least one
15 of the presence and concentration of the second component to develop an indication of the at least one of the presence and concentration of the second component, and removing an amount representative of the indicated presence or concentration of the second component from the concentration of the first component indicated by the first measurement.
2. The method of claim 1 wherein the second measurement is of a
20 largely glucose-insensitive measure of hematocrit.
3. The method of claim 1 or 2 wherein the first measurement is of a hematocrit-sensitive measure of glucose concentration.
4. The method of claim 1 further including contacting the biological fluid or control with a reactant before performing the first measurement.
- 25 5. Method according to claim 1, wherein said first measurement is a Cottrell current measurement.
6. Method according to claim 1 or 5, wherein said second measurement is a capacitance measurement of a test strip with sample applied to it.
7. Method according to claim 1, wherein a current is measured as
30 said first measurement and the deficit between said current and a Cottrell current is attributed to a capacitance, representing the presence or concentration of a second component, said current attributed to a capacitance being removed from the current measurement to determine the concentration of said first medically significant component.
- 35 8. Method according to claim 1 or 6, wherein said second measurement is made by using Linear Transient Analysis of said first measurement.

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5 9. The method of claim 1 wherein performing a first measurement on the biological fluid or control which first measurement varies with both the concentration of the first component and at least one of the presence and concentration of the second component

10 said method includes performing measurements of a time-varying function $i_1(t)$ having the general form

$$i_1(t) = M/\sqrt{t} + B$$

where t is time from initiating the measurement, M is the slope of a graph of the function and B is a value the function approaches as t becomes very large.

15 10. The method of claim 9, wherein the time varying function $i_1(t)$ is an electrical current, preferably the Cottrell current.

20 11. The method of claim 1 wherein performing a second measurement on the biological fluid or control which second measurement varies primarily only with the at least one of the presence and concentration of the second component to develop an indication of the at least one of the presence and concentration of the second component includes performing at least one measurement of a time-varying function $i_2(t)$, where t is time, $t <$ some arbitrarily established time.

12. Method according to claim 11, wherein $i_2(t)$ is an admittance.

25 13. The method of claim 1 wherein performing a first measurement on the biological fluid or control which first measurement varies with both the concentration of the first component and at least one of the presence and concentration of the second component, performing a second measurement on the biological fluid or control which second measurement varies primarily only with the at least one of the presence and concentration of the second component to develop an indication of the at least one of the presence and concentration of the second component, and removing an amount representative of the indicated presence or concentration of the second component from the concentration of the first component indicated by the first measurement together include performing measurements of a time-varying function $i_1(t)$ having the general form

$$i_1(t) = M/\sqrt{t} + B$$

35 where t is time from initiating the measurement, M is the slope of a graph of the function and B is a value the function approaches as t becomes very large, performing

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5 at least one measurement of $i_2(t)$, $t <$ some arbitrarily established time, and removing
from the measurements of $i_1(t)$ the at least one measurement of $i_2(t)$.

14. Apparatus for determining the concentration of a first,
medically significant component (e.g. glucose) of a biological fluid or a control (42),
10 the biological fluid or control (42) including a second component (e.g. blood cells)
which affects the determination of the concentration of the first component, the
apparatus including a device (20, 22) for performing a first measurement on the
biological fluid or control (42) which first measurement varies with both the
concentration of the first component and at least one of the presence and
15 concentration (e.g. hematocrit) of the second component, performing a second
measurement on the biological fluid or control (42) which second measurement varies
primarily only with the at least one of the presence and concentration of the second
component to develop an indication of the at least one of the presence and
concentration of the second component, and removing an amount representative of
20 the indicated presence or concentration of the second component from the
concentration of the first component indicated by the first measurement.

15. The apparatus of claim 14 for performing the first measurement
on the blood, blood fraction or control (42) which first measurement varies with both
the concentration of glucose and the concentration (e.g. hematocrit) of blood cells in
25 blood, a blood fraction or a control (42), the second measurement varies primarily
only with the concentration of blood cells in the blood, blood fraction or control (42).

16. The apparatus of claim 14 further including a reactant (e.g.
glucose oxidase) for contacting the biological fluid or control (42) before performing
the first measurement.

30 17. Apparatus according to claim 14, wherein said first
measurement is a Cottrell current measurement.

18. Apparatus according to claim 14 or 17, wherein said second
measurement is a capacitance measurement of a test strip with sample applied to it.

19. Apparatus according to claim 14, wherein said first
35 measurement is a current measurement and the deficit between said current and a
Cottrell current is attributed to a capacitance representing the presence or
concentration of a second component, said current being attributed to a capacitance

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5 and is removed from the current measurement to determine the concentration of said first medically significant component.

20. Apparatus according to claim 14 or 18, wherein said second measurement is made by using Linear Transient Analysis of said first measurement.

21. The apparatus of claim 14 wherein the device (20, 22) for
10 performing a first measurement on the biological fluid or control (42) which first measurement varies with both the concentration of the first component and at least one of the presence and concentration of the second component) includes a device (20, 22) for performing measurements of a time-
varying function $i_1(t)$ having the general form

15
$$i_1(t) = M/\sqrt{t} + B$$

where t is time from initiating the measurement, M is the slope of a graph of the function and B is a value the function approaches as t becomes very large.

22. The apparatus of claim 14 wherein the device (20, 22) for
performing a second measurement on the biological fluid or control (42) which
20 second measurement varies primarily only with the at least one of the presence and concentration of the second component to develop an indication of the at least one of the presence and concentration) of the second component includes performing at least one measurement of a time-varying function $i_2(t)$, where t is time, t being less than some arbitrarily established time

23. The apparatus of claim 14 wherein the device (20, 22)
removes from the measurements of $i_1(t)$ the at least one measurement of $i_2(t)$.

24. A method for substantially concurrently determining the
concentrations of first and second medically significant components of a biological
fluid or a control during an assay of a sample of the biological fluid or control, the
30 method including developing an equivalent electrical circuit model of a sample of the biological fluid or control and an apparatus for assaying the biological fluid or control, identifying at least a first element of the electrical circuit model, the behavior of which at least first element varies with the concentration of the first component relatively independently of the concentration of the second component, identifying at
35 least a second element of the electrical circuit model, the behavior of which at least second element

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5 - varies with the concentration of the second component relatively
independently of the concentration of the first component, or
 - varies with the concentrations of both the first and second components,
separately assessing the behaviors of the at least first element and the at least second
element during the assay, and determining from the separate assessments of the
10 behaviors of the at least first element and the at least second element the
concentrations of the first component and the second component.

25. Apparatus for substantially concurrently determining the
concentrations of first and second medically significant components of a sample of a
15 biological fluid or a control during an assay of the sample, the combination of the
apparatus for assaying the sample and the sample together characterized by an
equivalent electrical circuit model including at least a first element, the behavior of
which at least first element varies with the concentration of the first component
relatively independently of the concentration of the second component, the equivalent
20 electrical circuit model further including at least a second element, the behavior of
which at least second element

 - varies with the concentration of the second component relatively
independently of the concentration of the first component, or
 - varies with the concentrations of both the first and second components,
25 the apparatus including a device for separately assessing the behaviors of the at least
first element and the at least second element during the assay, and determining from
the separate assessments of the behaviors of the at least first element and the at least
second element the concentrations of the first component and the second component.

Fig. 1

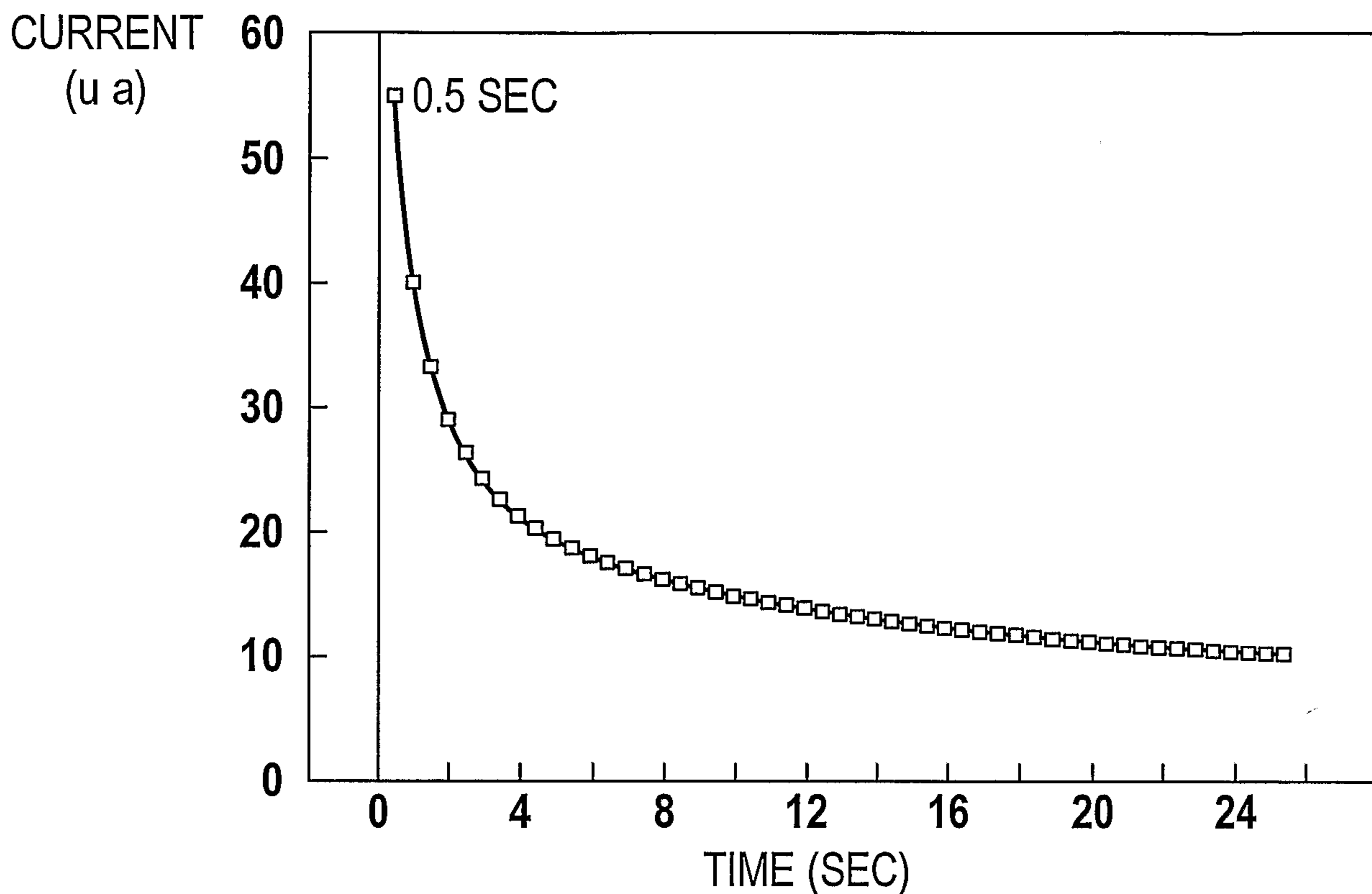


Fig. 2

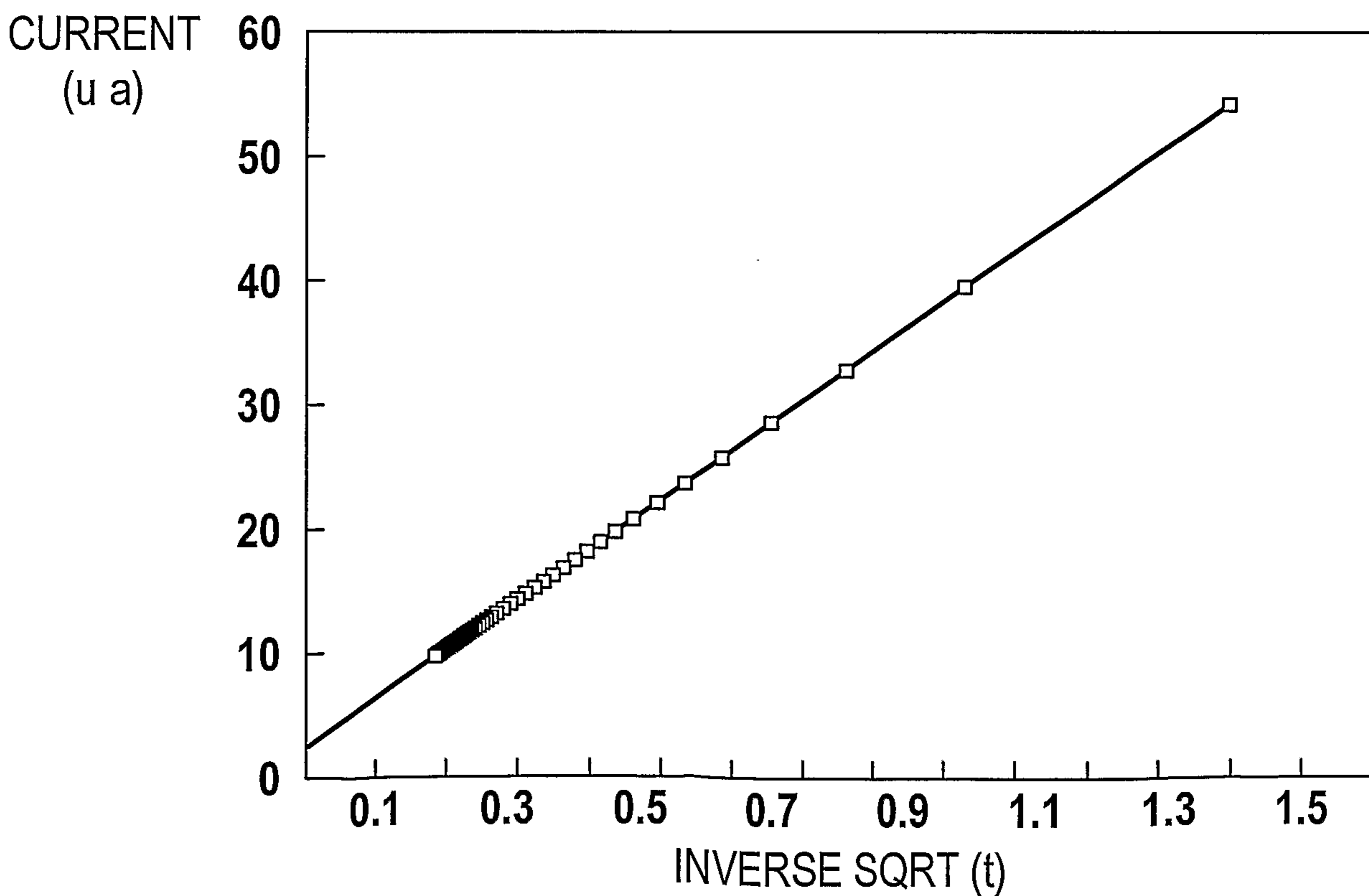


Fig. 3

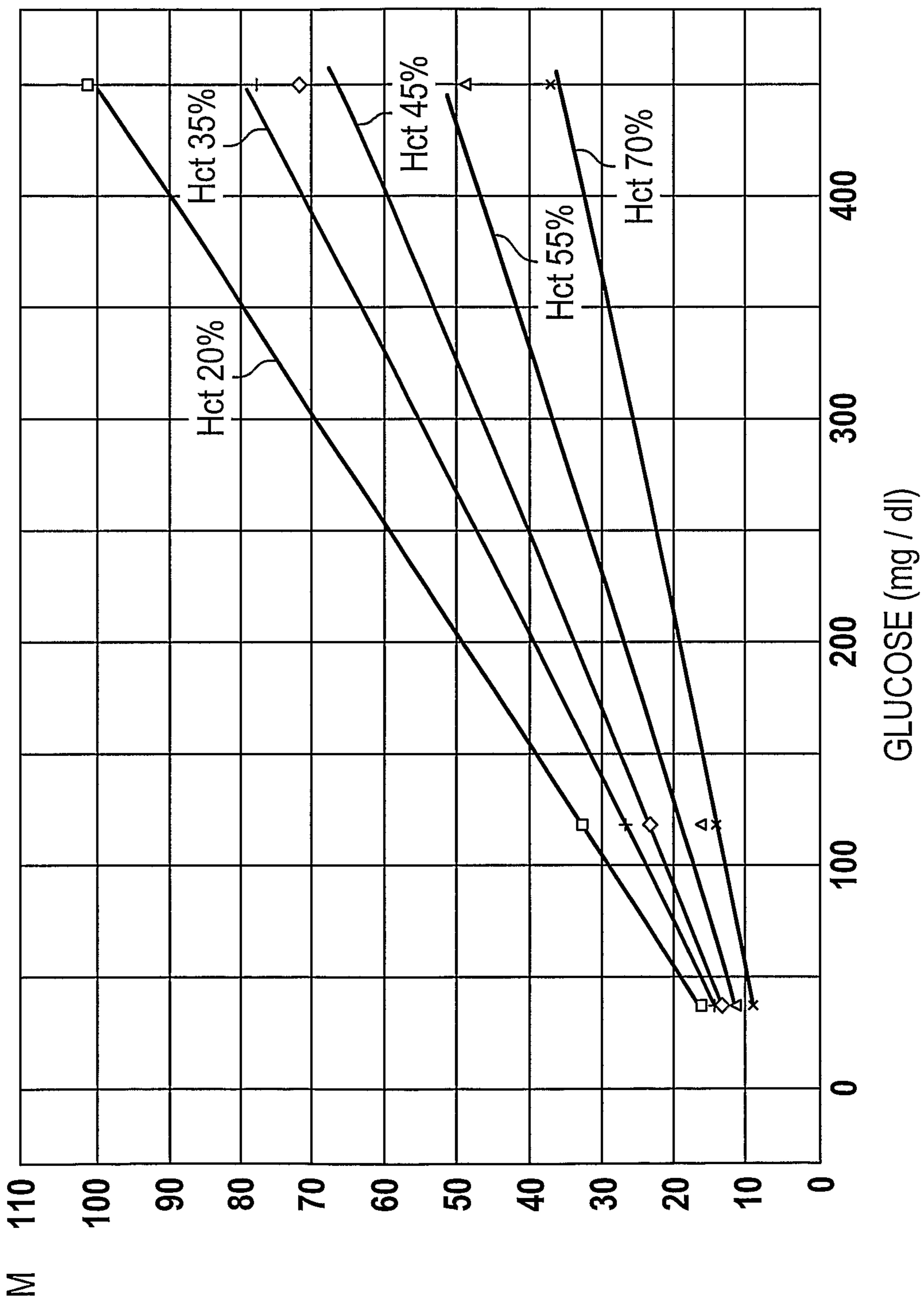


Fig. 4

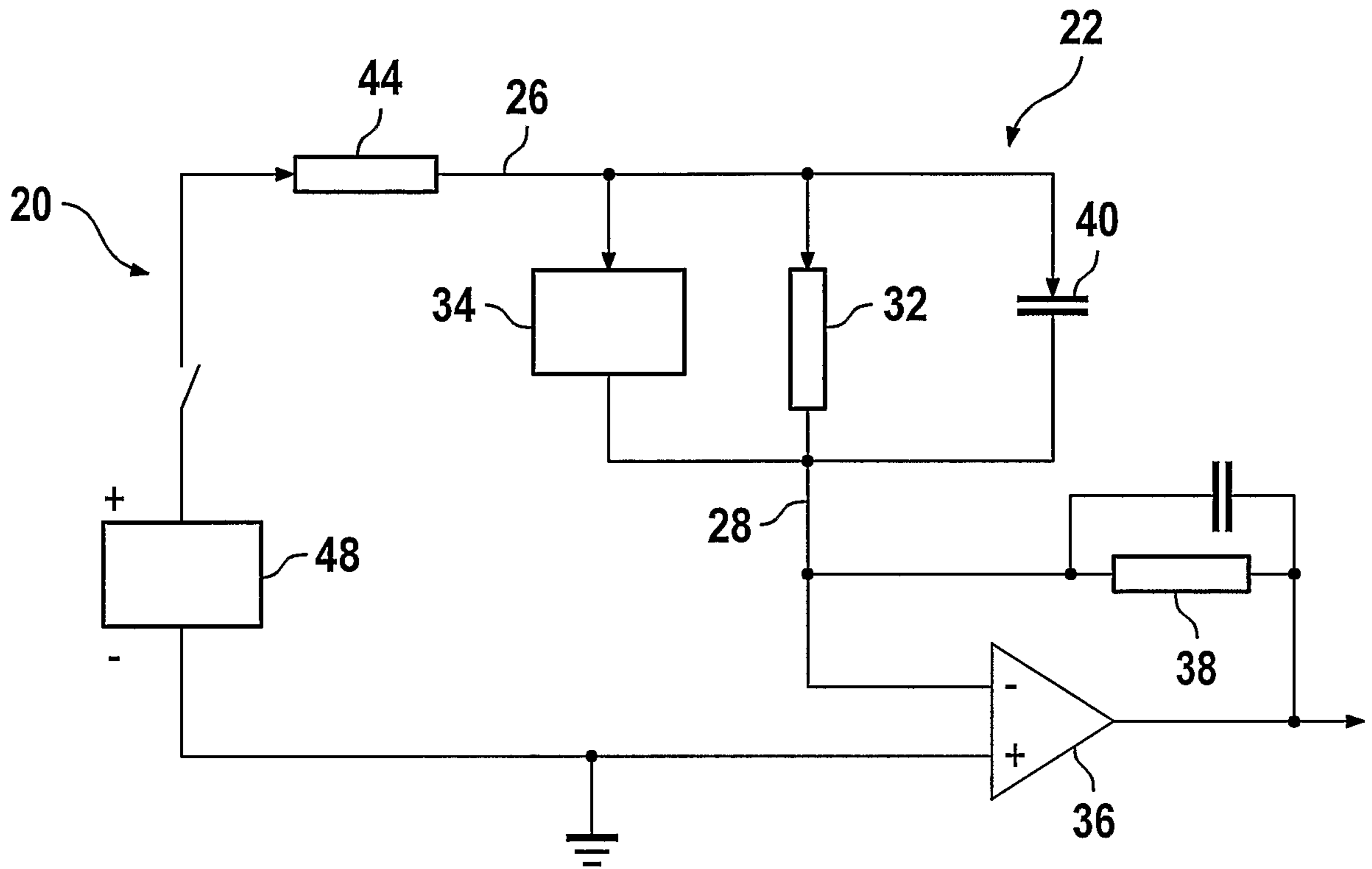
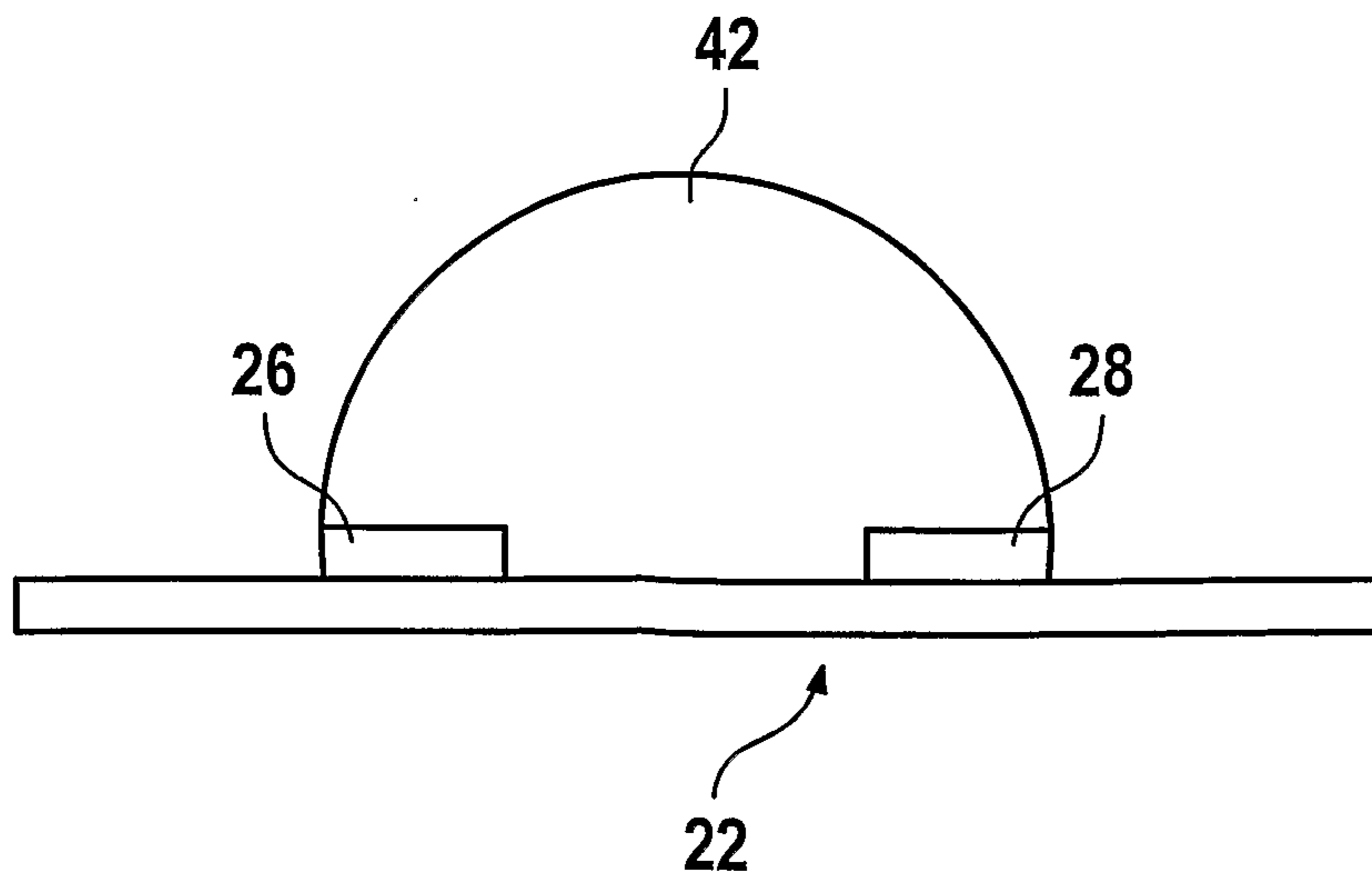
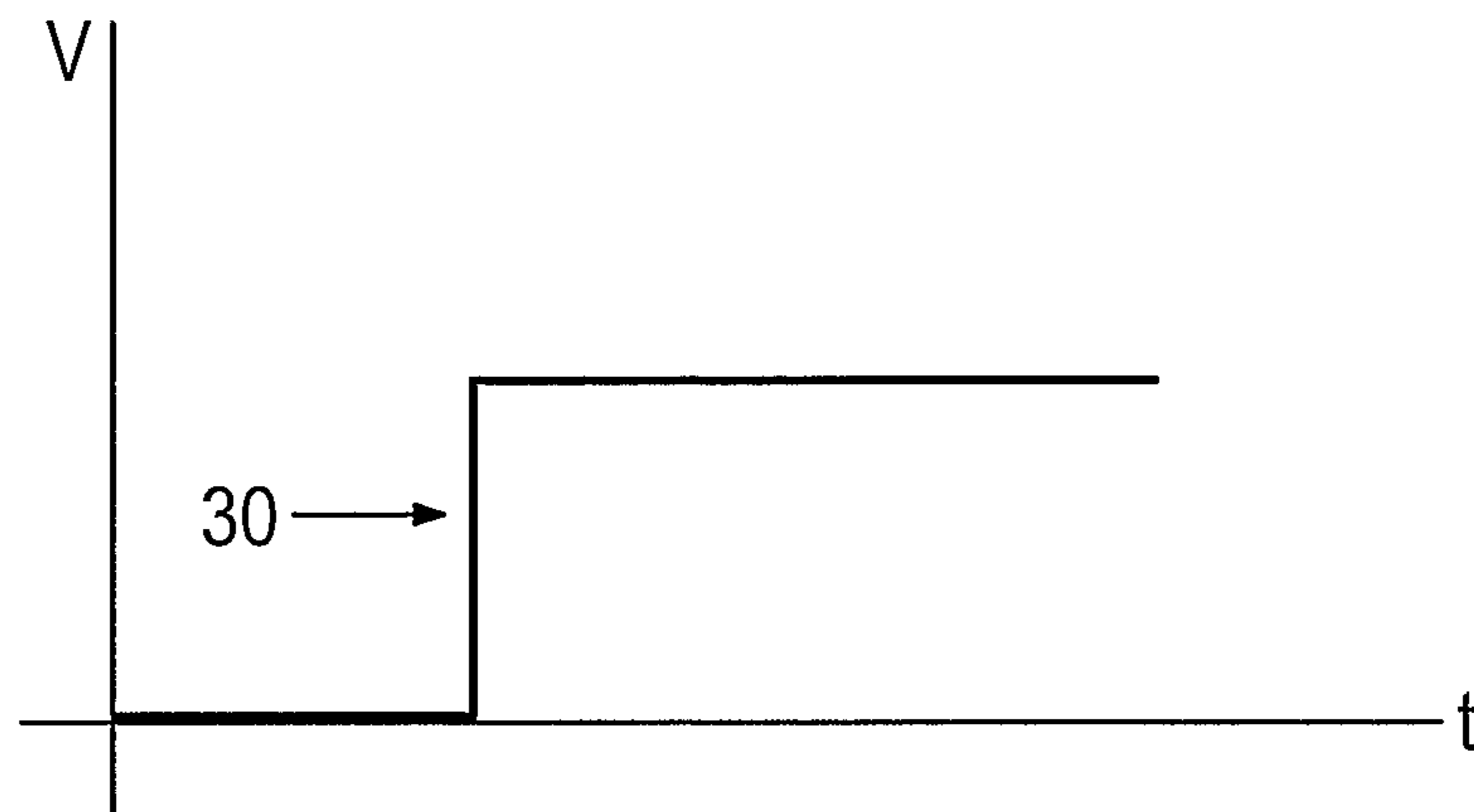
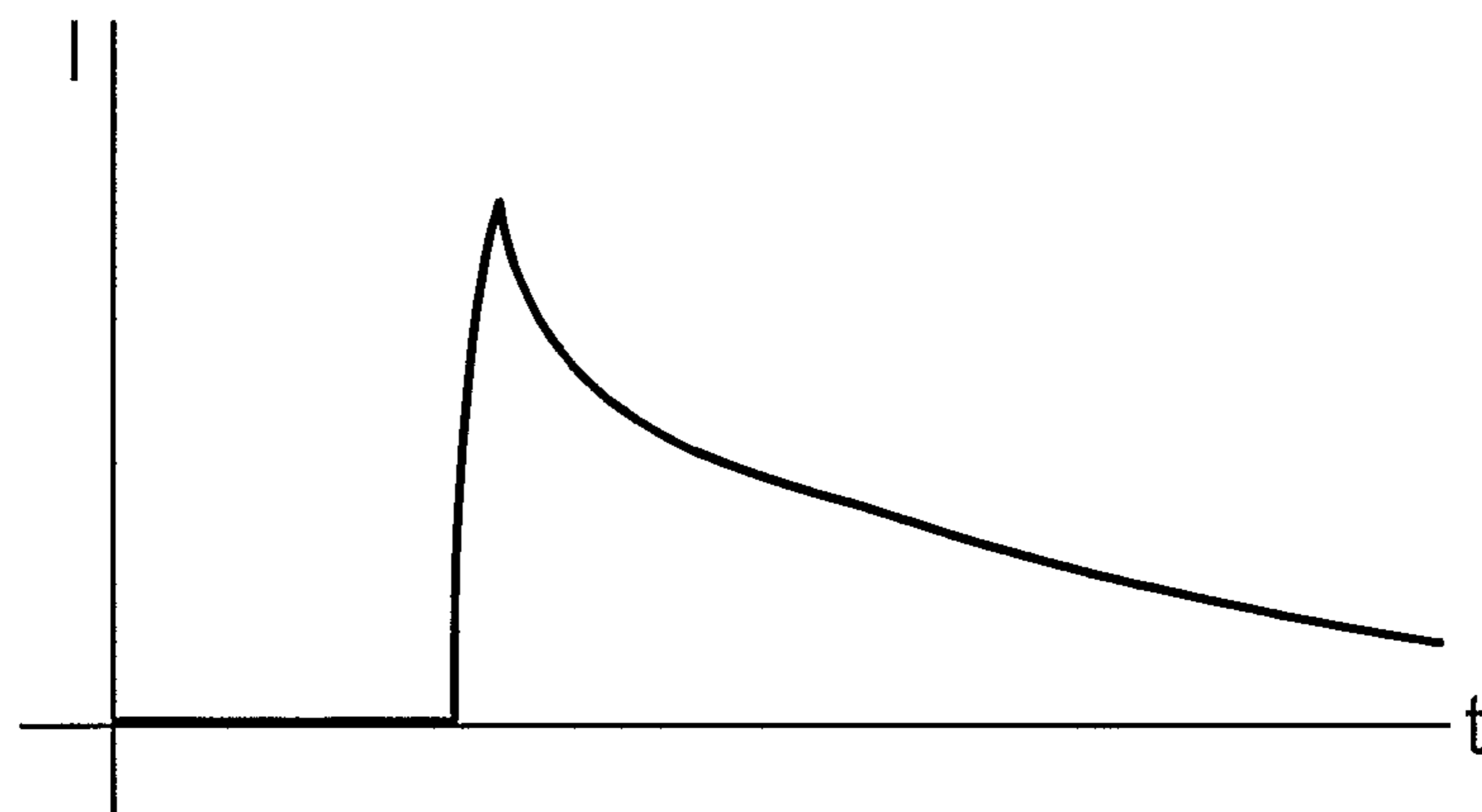
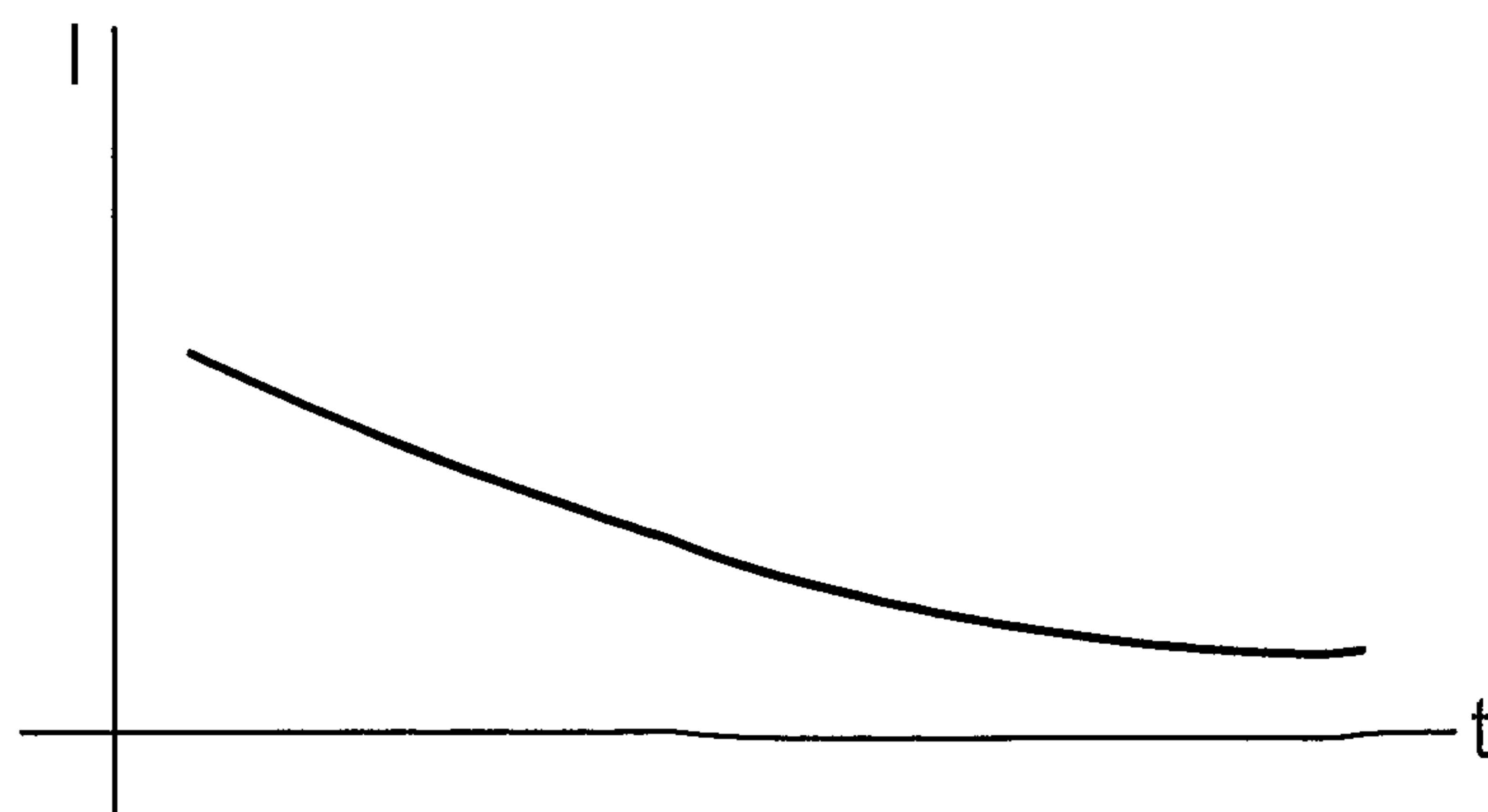


Fig. 5



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Fig. 6**Fig. 7****Fig. 8**

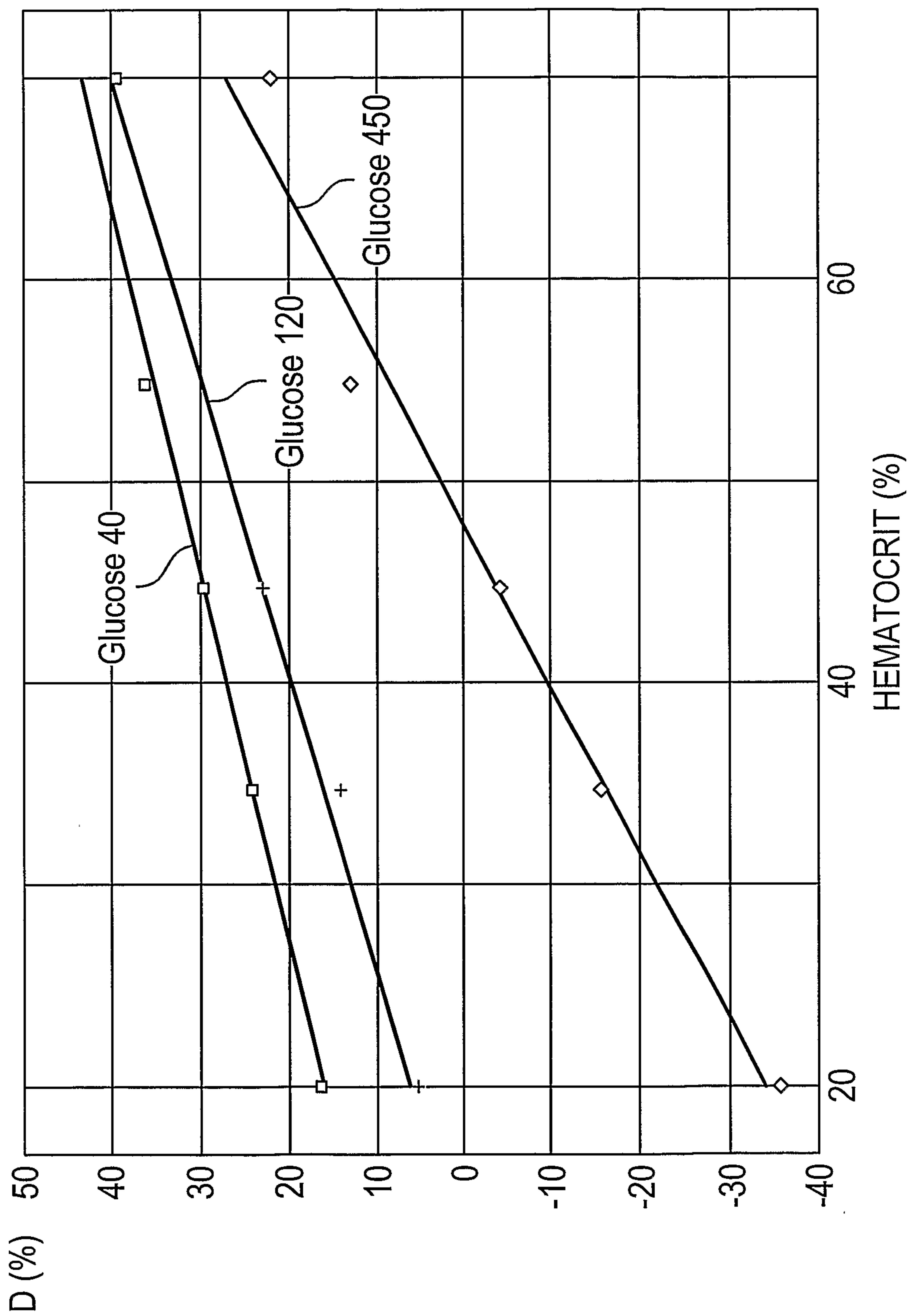
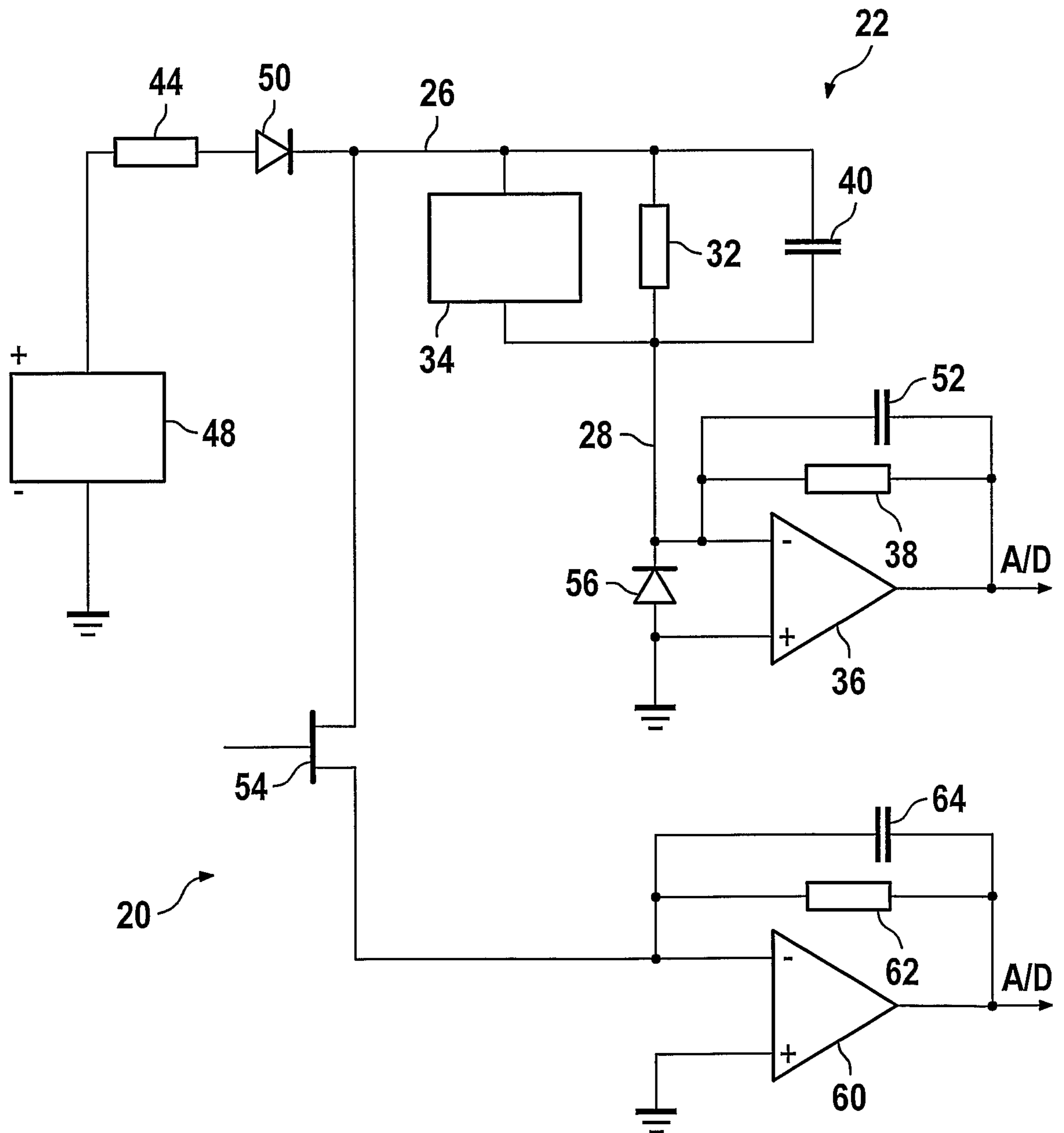


Fig. 9

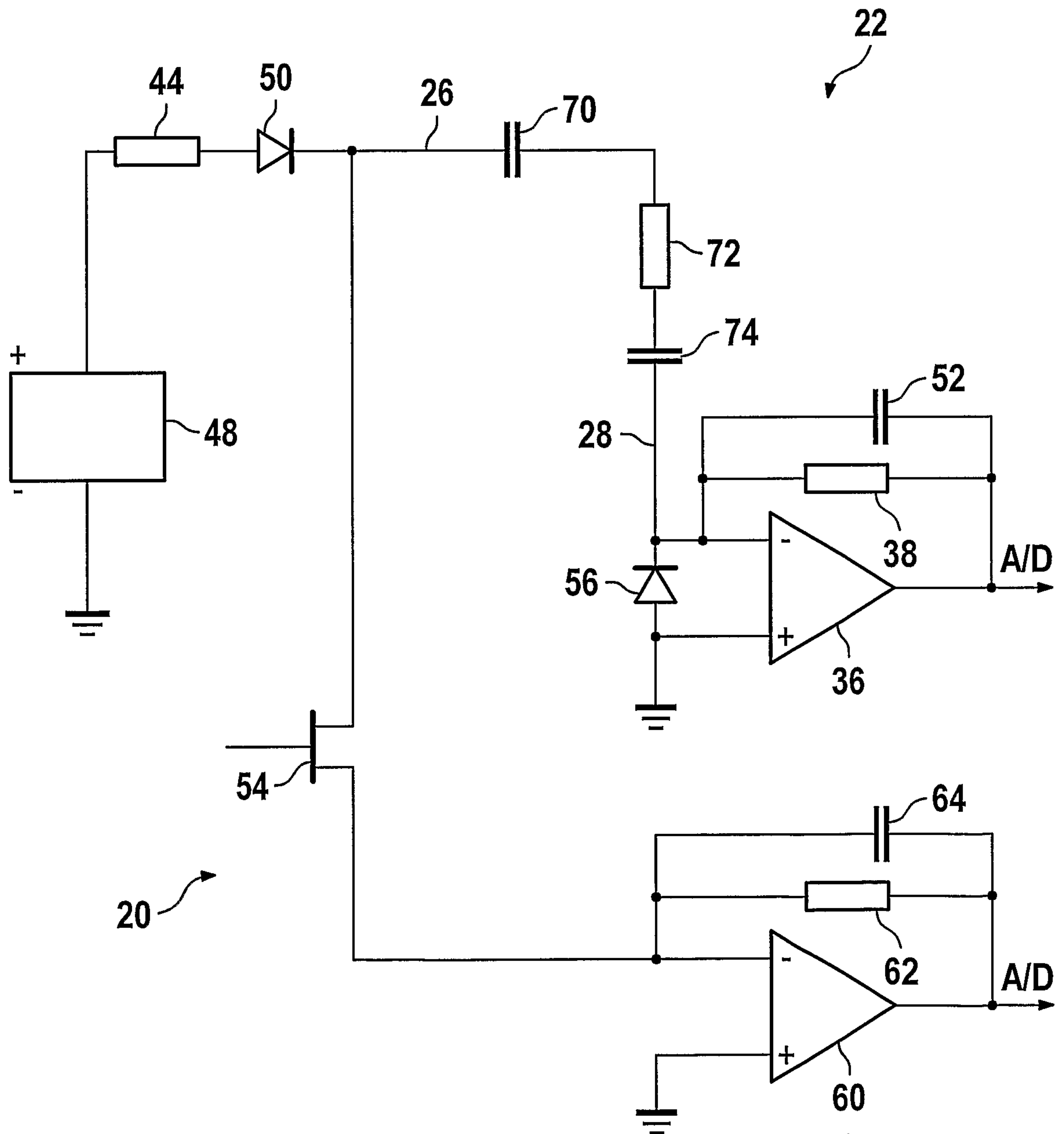
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Fig. 10



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Fig. 11



CURRENT
(u a)

