INSTRUCTIONS

(a) If Convention application insert "Convention"

(a) CONVENTION

AUSTRALIA 647626

Patents Act 1990

(b) Delete one

REQUEST FOR A (b) STANDARD/~~PETTY~~ PATENT

(c) Insert FULL name(s) of applicant(s)

~~I~~/We being the person(s) identified below as the Applicant, request the grant of a patent to the person identified below as the Nominated Person, for an invention described in the accompanying Standard/~~Petty~~(b) Complete Patent Specification.

~~I am~~/We are (b) not an opponent or eligible person described in Sections 33-36 of the Act.

[70,71] Applicant and Nominated Person (c): Toa Medical Electronics Co., Ltd.

(d) Insert FULL address(es) of applicant(s)

of (d) 2-1, Minatojima Nakamachi 7-chome, Chuoku, Kobe, Japan

(e) Insert TITLE of invention

[54] Invention Title (e) REAGENT FOR COAGULATING BLOOD

(f) Insert Names of actual Inventors.

[72] Names of Actual Inventors (f)

Masaru YONEMURA

[74] Address for Service:

PHILLIPS ORMONDE AND FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne, Australia 3000
Attorney Code: PO

(Note: The following applies only to Convention applications)

Basic Convention Application Details

(g) Insert number, country and filing date for the/or each basic application

(g) [31] Application No.	[33] Country	Country Code	[32] Date of Application
3-246740	Japan	JP	August 30,1991
Patent			

(h) Signature of applicant(s) (For body corporate see headnote*)

(i) Insert date of signing

(j) Corporate seal if any

Note: No legalization or other witness required

(j)

Reigo Hashimoto

(h) Signature of Applicant

May 12 1992

(i) Date

PHILLIPS ORMONDE AND FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne, Australia

P17/5/91

AUSTRALIA

Patents Act 1990

NOTICE OF ENTITLEMENT

INSTRUCTIONS
(a) Name of person making statement.

I (a) Reizo Hashimoto

(b) Position of that person.

(b) President

(c) Name of applicant

of (c) Toa Medical Electronics Co., Ltd.

(d) Address of applicant

of (d) 2-1, Minatojima Nakamachi 7-chome, Chuoku, Kobe, Japan

State the following:-

(e) Delete as necessary

1. The nominated person (applicant) is entitled to the grant of a patent
(e) (i) as assignee of the actual inventor(s)
~~(ii) by contract of employment of the actual inventor(s)~~
or (iii) (f)

(f) Insert details if not covered by (i) or (ii)

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2. The nominated person (applicant) is entitled to claim priority from the basic convention application~~(s)~~.
(g) (i) as applicants of the said application~~(s)~~
~~(ii) as the assignee of the applicants of the said application(s)~~
~~(iii) with the consent of the applicants of the said application(s)~~

(h) Delete for non-convention applications

3. The basic convention application~~(s)~~ was/~~were~~ the first made in a Convention country in respect of the invention the subject of the application. (h)

(i) Insert DATE of signing

Dated (i) May 12 1992

(j) Signature(s) of person making statement

(j) Reizo Hashimoto

Reizo Hashimoto

President

Note: No legalization or other witness required

To: The Commissioner of Patents

PHILLIPS ORMONDE AND FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne, Australia

P18/3/91

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REAGENT FOR COAGULATING BLOOD

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(71) Applicant(s)
TOA MEDICAL ELECTRONICS CO., LTD.

(72) Inventor(s)
MASARU YONEMURA

(74) Attorney or Agent
PHILLIPS ORMONDE & FITZPATRICK , 367 Collins Street, MELBOURNE VIC 3000

A reagent for coagulating blood for measuring blood coagulation ability containing coagulation factor activating substance such as tissue thromboplastin, phospholipid and thrombin, calcium ion, and high molecular substance such as high molecular vinyl and high molecular polysaccharide. By adding high molecular substance to the reagent, the turbidity change due to blood coagulation is increased, and hence the changing quantity of transmitted light or scattered light increase, thereby making it possible to detect more accurately. Besides, by adjusting the electric conductivity, pH and osmotic pressure to proper values, the turbidity change due to blood coagulation may be further amplified.

CLAIM

1. A reagent for coagulating blood comprising coagulation factor activating substance, calcium ion, and high molecular substance.

AUSTRALIA

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COMPLETE SPECIFICATION
(ORIGINAL)

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Name of Applicant:

Toa Medical Electronics Co., Ltd.

Actual Inventor(s):

Masaru Yonemura

Address for Service:

PHILLIPS ORMONDE & FITZPATRICK
Patent and Trade Mark Attorneys
367 Collins Street
Melbourne 3000 AUSTRALIA

Invention Title:

REAGENT FOR COAGULATING BLOOD

Our Ref : 291550
POF Code: 136233/93441

The following statement is a full description of this invention, including the best method of performing it known to applicant(s):

6006

SPECIFICATION

TITLE OF THE INVENTION

REAGENT FOR COAGULATING BLOOD

BACKGROUND OF THE INVENTION

The invention relates to a reagent for coagulating blood for measuring the coagulating activity of plasma specimen by using optical measuring apparatus or viscosity detecting apparatus, and more particularly to a reagent for coagulating blood capable of measuring specimen of low coagulating activity, while contributing to enhancement of accuracy in ordinary specimens, by preparing into a composition so that a larger optical changing quantity or viscosity changing quantity than ever may be obtained.

The mechanism of blood coagulation is explained by reference to Fig. 1. The mechanism of blood coagulation usually occurs in two pathways. One route is called the extrinsic coagulation, in which starting from the tissue thromboplastin released from epidermic cell or the like, the coagulated VII factor is activated, and by this activated coagulated VII factor, the coagulated X factor is activated, being followed by activation of the coagulated V factor, II factor, and finally fibrinogen is transformed into fibrin to induce coagulation.

The other route is called the intrinsic coagulation, in which the coagulated XII factor is activated by contact or the like to activate the XI factor. In succession, the activated XI factor activates the IX factor, and further the activated IX factor activates the X factor by collaborating action with calcium ion and VIII factor. It is later followed by activation of the V factor and II factor, and finally fibrinogen is transformed into fibrin to induce coagulation.

Methods for detecting blood coagulation may be roughly classified into the method of increasing viscosity of liquid along with coagulation of blood (viscosity detecting method), the method of detecting white turbidity along with coagulation of blood (turbidity detecting method), and their combined method. In the viscosity detecting method, a bar-shaped or spherical magnetic matter is injected into the plasma specimen, and a reagent for detecting coagulation is mixed, and the motion of the magnetic matter becomes dull due to coagulation, which is detected. This viscosity detecting method, however, largely differs in the result of measurement depending on the shape of the fibrin lumps which are final products of blood coagulation (that is, the quantity of fibrin or stiffness of coagulated state), and its fatal defect is that it is impossible to detect unless the viscosity is higher than a specific level. Besides, because

of the measuring principle of observing the motion of magnetic matter, it is affected by the intensity of magnetic matter.

The turbidity detecting method is to measure the coagulation only by mixing the plasma specimen and coagulating reagent, and it does not require magnetic matter or other charging. The method of detection is available in the transmitted light detecting method and scattered light detecting method. In these methods of detection, if the fibrinogen quantity is small, the change of transmitted light quantity or scattered light quantity can be detected, and it is hence free from the shortcoming known in the viscosity detecting method. However, in either detecting method, it is quite natural that the detection is more accurate if the changing quantity of light is greater, and the coagulating reagent to be used is desired to have such a characteristic as to express the changing quantity of light very largely.

Fig. 2 is a diagram for explaining the change of signal intensity obtained in the scattered light detecting method. The result of investigating the plasma coagulating process by optical detecting device (scattered light detecting method) is shown. In the diagram, point A is the moment of mixing the plasma and coagulating reagent, and afterwards the coagulation reaction progresses in multiple steps, and

as a stable fibrin is formed, a change in scattered light appears (point B in the diagram). As the formation of stable fibrin is progressed, the change of scattered light increases, but most fibrinogen is consumed, and the change of quantity of scattered light decreases, and the reaction terminates (point C). The coagulating time may be, for example as disclosed in the Japanese Laid-open Patent Sho. 59-203959, determined as time T until reaching the 50% scattered light quantity supposing the quantity of scattered light at time B to be 0% and the quantity of scattered light at time C to be 100%. Incidentally ΔH is the changing quantity of scattered light from start of coagulation reaction till its end.

To measure the pathway of extrinsic coagulation, the method known as Quick's single-stage method is most popular at the present. This measurement (examination) is generally called the PT (prothrombin time) method, which is capable of not only measuring comprehensively the activity of extrinsic coagulating factors, but also measuring individual extrinsic coagulating factors by using coagulating factor deficient plasma. Today, in the patients administered anticoagulants (such as warfarin) for the treatment of heart disease, this measuring method is important as the means for monitoring the effect of anticoagulants.

Most reagents used in this method are prepared by

grinding rabbit brain in the presence of acetone, dehydrating, drying and extracting tissue thromboplastin. The tissue thromboplastin differs in reactivity to extrinsic coagulating factors or yield depending on the extraction conditions, and various methods of extraction have been invented so far. For example, Ronald Bach et al. attempted to enhance the yield by solubilizing the tissue thromboplastin by using surface active agent (Triton X-100) (The J. Biological Chemistry, Vol. 256, No. 16, pp. 8324-8331, 1981). As a similar method, in the Japanese Patent Publication Sho. 63-56501, it is proposed to use surface active agent of cholic acid group at the time of tissue thromboplastin extraction, and concerning these methods, M. Hvatum and H. Prydz (Biochimica et Biophysica Acta, Vol. 130, pp. 92-101, 1966) and H. Gonmori and Takeda (Thrombos. Haemostas. Vol. 36, pp. 90-130, 1976) reported the method of preparing tissue thromboplastin in detail in their papers. Besides, the Japanese Patent Publication Sho. 58-43080 discloses an invention for preparing a useful tissue thromboplastin by maintaining the pH during extraction at the alkaline side.

Whichever the method of extraction may be selected, it is indispensable to contain tissue thromboplastin in the reagent used in the inspection method (prothrombin time method) known at Quick's single-stage method, and at the

same time the tissue thromboplastin must be uniform in the reactivity to extrinsic coagulating factors and must finally possess the action of transforming fibrinogen into fibrin.

For the conventional reagent for coagulating blood (hereinafter called PT reagent), it was the ultimate requirement to have an accurate reactivity to extrinsic coagulating factors and a capability of finally forming fibrin, and little attention has been paid to display of accurate result of measurement. Accordingly, it was forced to allow significant fluctuations of result of examination about coagulation.

The problem that the invention is to solve is to prepare such a composition as to enlarge the optical changing quantity in order to display the result of measurement accurately. As a result, not only a specimen of low coagulating activity can be measured, but also it contributes to enhancement of accuracy in normal specimens.

OBJECT AND SUMMARY OF THE INVENTION

It is hence a primary object of the invention to present a reagent for coagulating blood capable of increasing the optical changing quantity and viscosity changing quantity.

To achieve the above object, the reagent for coagulating blood of the invention comprises a coagulating factor

activating substance, calcium ion, and high molecular substance.

Examples of coagulating factor activating substance include the following.

(a) Reagent containing tissue thromboplastin

Prothrombin time reagent, reagent for measuring protein induced by vitamin K (reagent for measuring complex factor)

(b) Reagent containing phospholipid

Partial thromboplastin time reagent, activated partial thromboplastin time reagent

(c) Reagent containing thrombin

Reagent for measuring fibrinogen, AT-III measuring reagent

It is hence a feature of the invention that the coagulating factor activating substance is a substance selected from the group comprising tissue thromboplastin, phospholipid and thrombin.

An example of reagent containing tissue thromboplastin is explained below. To prepare a reagent containing tissue thromboplastin, tissues (brain, lung, placenta, etc.) of mammal animals (human, rabbit, cattle, horse, monkey, etc.) are treated with acetone to obtain acetone treated powder (hereinafter called acetone powder), and fractions containing tissue thromboplastin are extracted from the acetone powder, and calcium ions and high molecular substances are

added in the fractions.

An example of manufacturing process is illustrated.

I. The brain of a rabbit is taken out.

II. Preparation of acetone powder

The rabbit brain is mixed and ground in acetone. The mixture is filtered and dried.

III. Removal of blood components and foreign proteins

The acetone powder is mixed in an acidic solution, and is centrifuged to obtain sediment fraction. At this time, the floating fraction contains blood components and foreign proteins.

IV. Extraction of tissue thromboplastin

The sediment fraction is mixed and stirred in solution, and centrifuged, and a floating fraction containing tissue thromboplastin is collected.

V. Addition of high molecular substances

Calcium ions and high molecular substances are added to the fraction containing tissue thromboplastin.

VI. Freeze-drying

The above reagent is freeze-dried.

At step II, meanwhile, it is desired to mix the rabbit brain and acetone at a ratio by weight of 1:7. The acetone powder is commercially available and may be bought.

Besides, at step III, by mixing acetone powder to an acidic solution (such as hydrochloric acid water), the

undesired component may be much eluted in the solution, so that the removal rate of undesired component may be enhanced (blood corpuscles hemolyze in acidity).

At step IV, by using sodium formate or sodium acetate at a concentration of 25 to 250 mM is used, a tissue thromboplastin of high coagulating activity may be obtained efficiently.

The high molecular substances at step V may include, among others, high molecular vinyl selected from the group comprising polyethylene glycol, polyvinyl alcohol, and polynoxylene, or high molecular polysaccharide selected from the group comprising agarose, soluble starch, glycogen, dextran and dextrin. As the high molecular substance, either high molecular vinyl or high molecular polysaccharide, or both may be used. More specifically, for example, polyethylene glycol with molecular weight of 1,000 to 500,000 (more preferably 5,000 to 100,000, most preferably 20,000), or dextran with molecular weight of 5,000 to 2,000,000 (more preferably, 50,000 to 500,000, or most preferably 170,000 to 220,000) may be preferred. The content may be properly determined, but it is generally at 0.25% to 2.0% (w/v), being that the effect of high sensitivity is not obtained if too little, or the reaction performance deteriorates due to excessive viscosity if too much.

After adding the high molecular substance, by setting

the reagent in the state at least satisfying one of the following conditions 1 to 3, the reagent of a higher sensitivity will be obtained (best when all three conditions are satisfied).

1. The electric conductivity is adjusted to 4.0 to 15 mS (more preferably 6.5 to 11.0 mS).

2. The pH is adjusted to 6.7 to 8.2 (more preferably 7.2 to 7.5).

3. The osmotic pressure is adjusted to 100 to 700 mOsm/kgcm^2 (more preferably 350 to 700 mOsm/kgcm^2).

For adjustment of electric conductivity, an electrolyte may be used (such as salt). For adjustment of pH, acid or alkali is used (e.g. hydrochloric acid, sodium hydroxide). For adjustment of osmotic pressure, a substance which is non-electrolytic and water-soluble at the same time is used (e.g. sugar, urea). The prepared thromboplastin reagent should be preferably freeze-dried so as to be stored for a long period. Before use, the dry powder is dissolved in a specific amount of water to return to an initial state of solution.

It is thus preferred in the invention to use, as the coagulating factor activating substance, a fraction containing tissue thromboplastin taken out from the acetone treated powder obtained by treating mammal tissues with acetone. The fraction containing tissue thromboplastin is preferably

obtained by dissolving the acetone treated powder in solution, mixing, stirring and centrifuging, or by floating the acetone treated powder in an acidic liquid, removing blood components and foreign proteins by centrifugation, adding liquid to the sediment fraction, stirring and then centrifuging.

The reagent containing tissue thromboplastin is described above, while the reagent containing phospholipid or the reagent containing thrombin may be obtained by employing the known art. Or as the reagent containing tissue thromboplastin, the prothrombin time reagent (PT reagent, the reagent for comprehensively measuring the extrinsic coagulation reaction in Fig. 1) is distributed by DADE, ORTHO, GD, all of the United States, Bering and BM, both of Germany, and others. As the reagent for measuring the activity of vitamin K derived enzyme (reagent for measuring complex factor), Thrombo Test and Hepaplastin Test (both tradenames) are distributed by Eisai of Japan, and Complex Factor II and Complex Factor T (both tradenames) are distributed by Kokusai Shiyaku of Japan.

As the reagent containing phospholipid, the APTT reagent and PTT reagent (the reagent for comprehensively measuring the intrinsic coagulation reaction in Fig. 1) are distributed by DADE, ORTHO, GD, all of the United States, Bering and BM, both of Germany, and others.

As the reagent containing thrombin, the reagent for measuring fibrinogen, reagent for measuring thrombin time, or reagent for measuring AT-III are distributed by DADE, ORTHO, GD, all of the United States, Bering and BM, both of Germany, Kokusai Shiyaku of Japan, and others.

Therefore, in the invention, as the coagulating factor activating substance, the phospholipid containing fraction obtained from acetone treated powder prepared by treating mammal tissue with acetone, or the thrombin containing fraction refined from plasma of mammal may be used.

By mixing the blood coagulation reagent of the invention in the plasma, coagulation factors in the plasma are activate one after another, and finally fibrinogen is transformed into fibrin to terminate the coagulation reaction. In the final process, first monomers of fibrin are formed, and the monomers are gradually polymerized to form polymer (fibrous), so that the fibrin become fibrous. As the fibrin fibers are entangled like a mesh, the mixed solution becomes turbid and is gelled.

By tracing the optical characteristics (for example, intensity of scattered light) of the mixed solution sequentially from the point of mixing the reagent, the time until end of coagulation is known, from which the coagulation ability is calculated.

The reagent of the invention contains high molecular

substance. By the coexistence of the fiber and high molecular substance, the entangling is more complicated, and the difference in optical characteristic between right after mixing the reagent (before coagulation) and after coagulation reaction is greater than before (that is, higher in sensitivity).

Besides by varying the electric conductivity, pH or osmotic pressure, the quantity of optical change varies. By setting these values at proper values, a higher sensitivity is expected.

Thus, when the reagent of the invention is mixed with the specimen plasma, the coagulation factors in the plasma are sequentially activated, and finally the fibrinogen is transformed into fibrin. At this time, for example, by detecting the turbidity change (increase of turbidity) optically, the coagulation time is measured, from which the coagulation ability is assessed (calcutated).

In the final process of blood coagulation, the fibrinogen is transformed into fibrin, but initially monomers of fibrin are formed. As the reaction is advanced, the fibrin monomers grown into dimer and further into polymer (fiber) by polymerization reaction. In this process, the greater the quantity of fibers (fibrin) and the more complicated is the fiber entanglement, the greater is the turbidity change, and therefore the measurement of coagulation ability is

enhanced in both accuracy and reproducibility. The blood coagulation reagent of the invention contains the high molecular substance in order to cause a greater change in the turbidity, and therefore it increases the hardness of coagulation owing to entangling of fibers and high molecular substance, as well as entangling of fibers, and amplifies the optical change quantity. Besides, by properly controlling the conditions such as electrical conductivity, the polymerization reaction from fibrin monomers to fibrin polymer may be encouraged. It is anyway effective to increase the turbidity change due to blood coagulation, so that a reagent of high sensitivity may be prepared.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a block diagram showing a general mechanism of blood coagulation.

Fig. 2 is a graph showing the relation between change of signal and blood coagulation obtained by the conventional method of detecting scattered light.

Fig. 3 is a graph showing the relation between the quantity of scattered light and time in a reagent for coagulating blood of the invention and a conventional reagent for coagulating blood not containing high molecular substance.

Fig. 4 is a graph showing the relation between the electric conductivity and change of scattered light.

Fig. 5 is a graph showing the relation between pH and the change of scattered light.

Fig. 6 is a graph showing the relation between the osmotic pressure and change of scattered light.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

For the reagent used in the method of examination called Quick's single-stage method (prothrombin time method), it is indispensable to contain tissue thromboplastin in order to endow the extrinsic coagulation factors with reactivity, and moreover in order that the result of reaction be measured accurately, it is necessary to set the reaction environments so that the coagulation lumps may be firmer. In an embodiment, accordingly, the tissue thromboplastin of high reactivity was extracted from the rabbit brain tissues in the following procedure.

(1) The rabbit was anesthetized with chloroform or other anesthetic, and the whole blood was extracted from the cervical artery. From the brain artery, normal (physiological) saline was perfused to rinse the blood in the brain. Then the cranium was opened, and the brain was removed (picked out).

(2) To 100g of rabbit brain obtained in this way, 700 ml of cold acetone was mixed, and the brain was ground and stirred by a mixer, and dehydrated and pulverized. The

ground matter was filtered and dried in vacuum, and the acetone powder (AP) was obtained.

(3) 10g of AP was mixed in 100 ml of hydrochloric acid water (0.02 normal), and was centrifuged (3,000 rpm × 30 min) to collect sediment fractions. The separated and removed floating fractions contained blood components and foreign proteins.

(4) The collected sediment fractions were mixed and stirred in 100 ml of sodium formate (0.05 normal). By centrifugation (3,000 rpm × 30 min), floating fractions containing tissue thromboplastin were collected.

(5) To the collected floating fractions containing about 100 ml of tissue thromboplastin, 50 mM buffer agent for stabilizing the pH (phosphoric acid buffer solution, barbital buffer solution or tris buffer solution may be also usable), preservatives (0.1% w/v sodium azide), and stabilizer (0.1% calcium chloride) were mixed, and in order to make the coagulation reaction more manifest, in addition, a reagent was mixed, such as 0.5% polyethylene glycol (molecular weight 20,000) or 0.1% dextran (molecular weight 200,000). Furthermore, using sodium hydroxide of 0.1 normal, the pH was adjusted to 7.20 to 7.50, and the salt was mixed to adjust the electric conductivity to 6.5 to 10.0 mS, and glucose was added to adjust the osmotic pressure to 350 to 700 mOsm/kgcm^2.

(6) Thus obtained reagent containing tissue thromboplastin was freeze-dried and stored.

The effect of removing blood components and foreign proteins by treating the acetone powder is explained. Before extracting the tissue thromboplastin, the acetone powder was dissolved in solution, and in order to investigate the effect of removing the blood components and foreign proteins, the deproteinized specimen (operation to wash acetone powder in acidic solution) and the control specimen (not deproteinized) were prepared, and using these PT reagents, ten measurements each were compared. As a result, as shown in Table 3, the deproteinized PT reagent was higher in coagulation activity than the control PT reagent without this treatment (that is, the coagulation time was shorter), and was superior in reproducibility.

It is hence known that the operation to wash the acetone powder in acidic solution prior to extraction of tissue thromboplastin is effective for enhancing the coagulation activity of PT reagent and for obtaining results of higher precision.

Table 1

	Coagulation time	Reproducibility (variation factor)
Deproteinized	14.58 sec	0.52%
Not deproteinized	16.52 sec	1.86%

Still more, in order to see the effect of mixing high molecular substance in the reagent, the PT reagent containing tissue thromboplastin blended with high molecular substance and the PT reagent containing tissue thromboplastin without blend were prepared, and their coagulation curves were compared. As a result, as shown in Fig. 3, the PT reagent with high molecular substance was greater in the quantity of change of scattered light as compared with the PT reagent without the blend
($\Delta H > \Delta H_1$).

It is hence known that the coagulation reaction can be analyzed more closely and precisely by mixing high molecular substance to the PT reagent. As the high molecular substance, ethylene glycol with molecular weight of 20,000 was used, and the blending rate was 0.5 w/v%. Meanwhile, ΔH denotes the change of scattered light by adding high molecular substance, and ΔH_1 represents the change of scattered light without high molecular substance.

Fig. 4 shows the effect of adjusting the electric

conductivity to 4.0 to 15.0 mS. To investigate the effect of adjusting the electric conductivity of the PT reagent to 4.0 to 15.0 mS, salt was added to tissue thromboplastin, and the PT reagent were prepared so that the electric conductivity be 2.0 to 20.0 mS, and using these PT reagents the plasma coagulation was measured, and the mean of changing quantity of scattered light (the mean of ten measurements each) was compared. As a result, as shown in Fig. 4, although the changing quantity of scattered light tended to decline as the electric conductivity elevated, there was a stabilizing tendency between 4.0 and 15.0 mS.

Hence, by adjusting the electric conductivity of the PT reagent to 4.0 to 15.0 mS, it is known that a stable change of scattered light may be obtained even if the electric conductivity varies depending on the plasma to be measured, while maintaining a high level of change of scattered light.

Fig. 5 shows the effect of adjusting the pH to 6.7 to 8.2, or preferably 7.2 to 7.5. To investigate the effect of adjusting the pH of the PT reagent to 6.7 to 8.2, or preferably 7.2 to 7.5, PT reagents were prepared in a pH range of 6.7 to 8.5 by using hydrochloric acid of 1 normal and sodium hydroxide of 1 normal in tissue thromboplastin extract, and the plasma coagulation was measured by using these PT reagents, and the mean of changing quantity of scattered light (the mean of ten measurements each) was compared. As a

result, as shown in Fig. 5, at the pH of 6.7 to 7.5, the changing quantity of the scattered light tended to be stable at a relatively high level, but between pH 7.5 and 8.2, as the pH elevated, the changing quantity of scattered light tended to decline, and at pH 8.2 or higher, the changing quantity of scattered light tended to be stable at a relatively low level.

Hence, by adjusting the pH of the PT reagent within 6.7 to 8.2, or preferably 7.2 to 7.5, it is known that a stable changing quantity of scattered light is obtained regardless of fluctuations of pH depending on plasma specimens, while maintaining the changing quantity of scattered light at high level.

Fig. 6 shows the effect of adjusting the osmotic pressure to 100 to 700 mOsm/kgcm^2, or preferably 350 to 700 mOsm/kgcm^2. To see the effect of adjusting the osmotic pressure of PT reagent to 100 to 700 mOsm/kgcm^2, or preferably 350 to 700 mOsm/kgcm^2, glucose was mixed to tissue thromboplastin extract, and reagents were prepared by adjusting the osmotic pressure in a range of 50 to 900 mOsm/kgcm^2, and using these PT reagents, the plasma coagulation was measured, and the mean of the changing quantity of scattered light at that time (the mean of ten measurements each) was compared. As a result, as shown in Fig. 6, the changing quantity of scattered light tended to decline along

with the elevation of osmotic pressure, but at the osmotic pressure of 100 to 700 mOsm/kgcm^2, or in particular in a range of 350 to 700 mOsm/kgcm^2, the changing quantity of scattered light tended to be stable.

Hence, by adjusting the osmotic pressure of the PT reagent to 100 to 700 mOsm/kgcm^2, or preferably 350 to 700 mOsm/kgcm^2, it is known possible to obtain a stable changing quantity of scattered light regardless of fluctuations of the osmotic pressure depending on specimen plasma, while maintaining the changing quantity of scattered light at a high level.

The reagent for coagulating blood of the invention possesses reactivity to coagulation factors, same as the conventional coagulation factor activating reagent, and contains high molecular substance, and therefore it is effective to increase the turbidity change along with blood coagulation. By properly setting the conditions such as electric conductivity, the polymerization reaction from fibrin monomers to fibrin polymer can be encouraged, and it is also effective to increase the turbidity change along with blood coagulation.

These facts suggest that the formation state of fibrin lumps as the final product of blood coagulation is firm, and therefore in the measuring apparatus on the basis of the principle of detection of viscosity, the detectable range is

extended even if the fibrinogen quantity is small, while the demerit of being influenced by intensity of magnetic substance or the like may be solved. In the turbidity detection method, the changing quantity of transmitted light or scattered light increases, so that a more accurate detection may be realized.

The description herein relates to the field of blood coagulation, but the method and reagent for adjusting the coagulation reaction environments and making the reaction manifest are not limited to the reagent used in the coagulation factor activating substance disclosed in the invention, but may be applied to everything that reacts to solidify or coagulate physically.

What is claimed is:

The claims defining the invention are as follows:

1. A reagent for coagulating blood comprising coagulation factor activating substance, calcium ion, and high molecular substance.

2. A reagent for coagulating blood of claim 1, wherein the high molecular substance is at least one selected from the group comprising high molecular vinyl and high molecular polysaccharide.

3. A reagent for coagulating blood of claim 1, wherein the high molecular substance is a high molecular vinyl selected from the group comprising polyethylene glycol, polyvinyl alcohol, and polynoxyline.

4. A reagent for coagulating blood of claim 1, wherein the high molecular substance is a high molecular polysaccharide selected from the group comprising agarose, soluble starch, glycogen, dextran and dextrin.

5. A reagent for coagulating blood of claim 1, wherein the coagulation factor activating substance is a substance selected from the group comprising tissue thromboplastin, phospholipid and thrombin.

6. A reagent for coagulating blood of claim 1, wherein the coagulation factor activating substance is fraction containing tissue thromboplastin taken out from acetone treated powder obtained by treating mammal tissues with acetone.

7. A reagent for coagulating blood of claim 1, wherein the

coagulation factor activating substance is a fraction containing phospholipid taken out from acetone treated powder obtained by treating mammal tissues with acetone.

8. A reagent for coagulating blood of claim 1, wherein the coagulation factor activating substance is a fraction containing thrombin refined from mammal plasma.

9. A reagent for coagulating blood of claim 6, wherein the fraction containing tissue thromboplastin is obtained by dissolving acetone treated powder in solution, mixing, stirring, and centrifuging.

10. A reagent for coagulating blood of claim 6, wherein the fraction containing tissue thromboplastin is obtained by floating acetone treated powder in acidic solution, centrifuging to remove blood components and foreign proteins, adding solution to the sediment fraction, stirring, and centrifuging.

11. A reagent for coagulating blood of claim 6, 9, or 10, wherein the electric conductivity is adjusted to 4.0 to 15.0 mS.

12. A reagent for coagulating blood of claim 6, 9, or 10, wherein the pH is adjusted to 6.7 to 8.2.

13. A reagent for coagulating blood of claim 6, 9, or 10, wherein the osmotic pressure is adjusted to 100 to 700 $mOsm/kgcm^2$.

14. A reagent for coagulating blood according to any one of

claims 1 to 6, 8 to 13, wherein the reagent is freeze-dried after adjustment.

DATED: 3rd June, 1992

PHILLIPS ORMONDE & FITZPATRICK

Attorneys for:

TOA MEDICAL ELECTRONICS CO., LTD.

David B Fitzpatrick

ABSTRACT OF THE DISCLOSURE

A reagent for coagulating blood for measuring blood coagulation ability containing coagulation factor activating substance such as tissue thromboplastin, phospholipid and thrombin, calcium ion, and high molecular substance such as high molecular vinyl and high molecular polysaccharide. By adding high molecular substance to the reagent, the turbidity change due to blood coagulation is increased, and hence the changing quantity of transmitted light or scattered light increase, thereby making it possible to detect more accurately. Besides, by adjusting the electric conductivity, pH and osmotic pressure to proper values, the turbidity change due to blood coagulation may be further amplified.

FIG.1 PRIOR ART

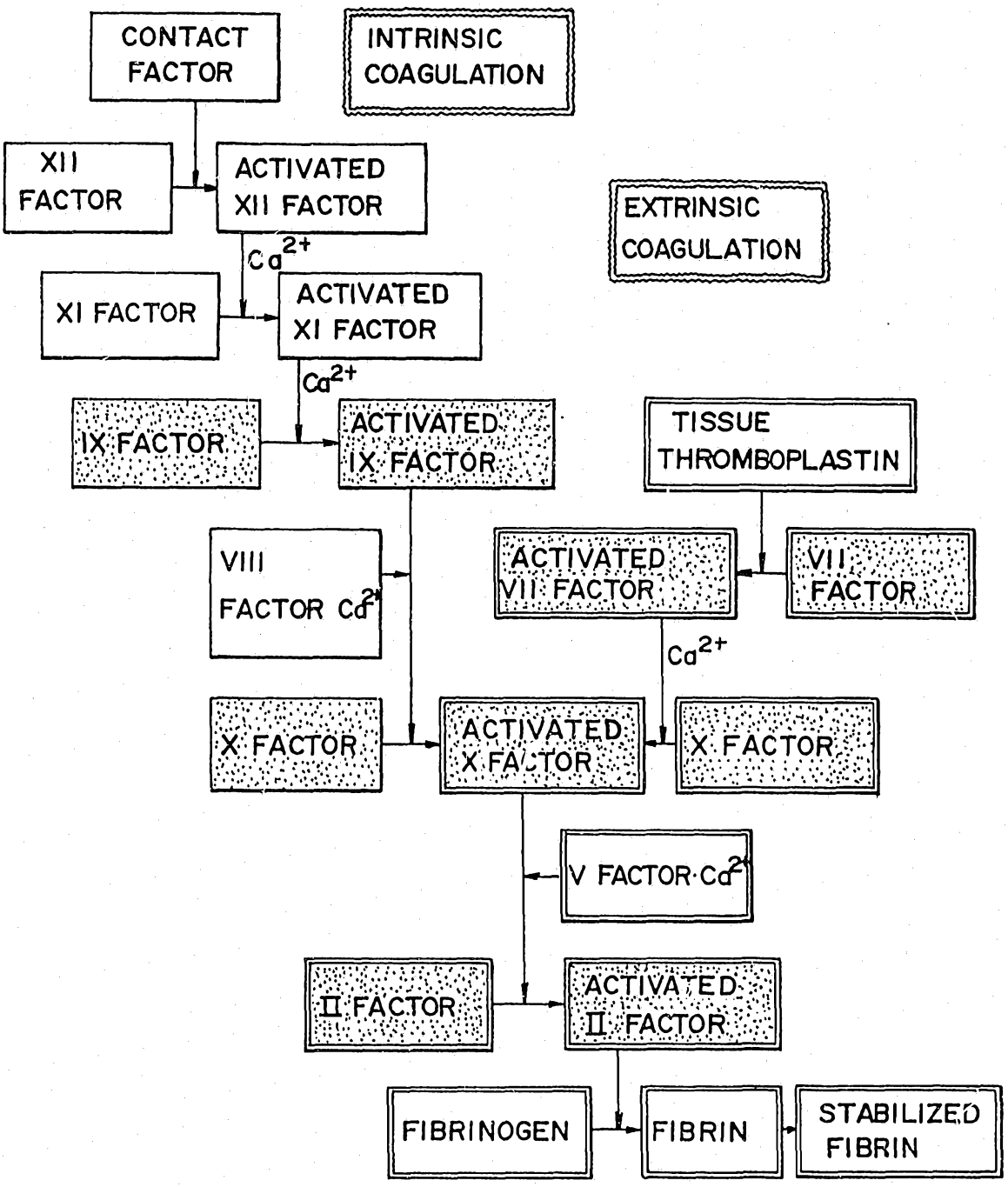


FIG. 2 PRIOR ART

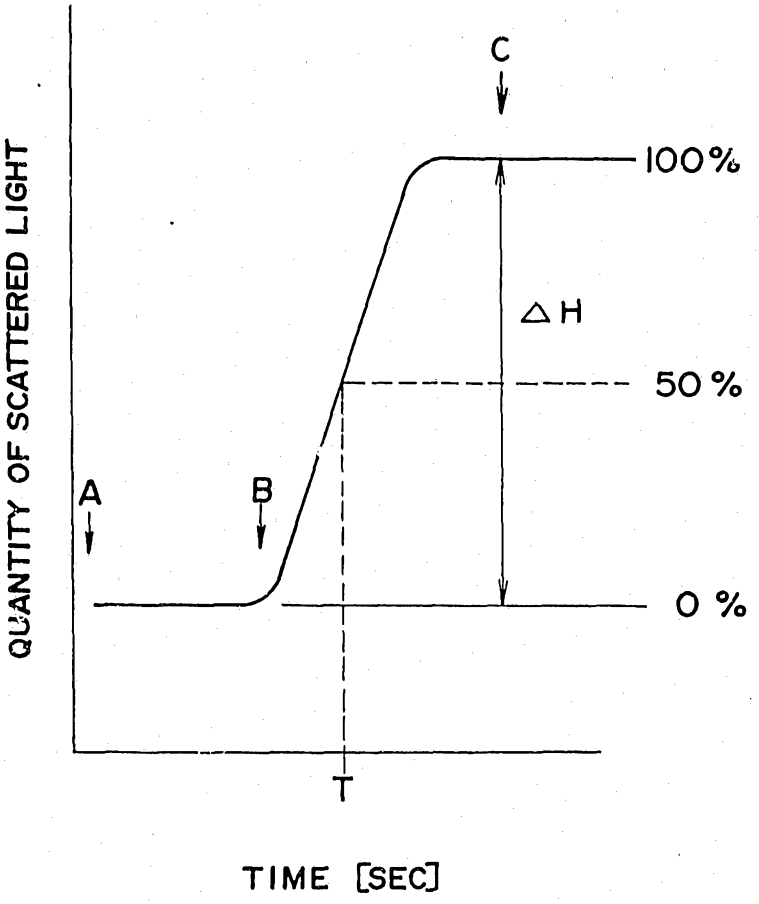


FIG. 3

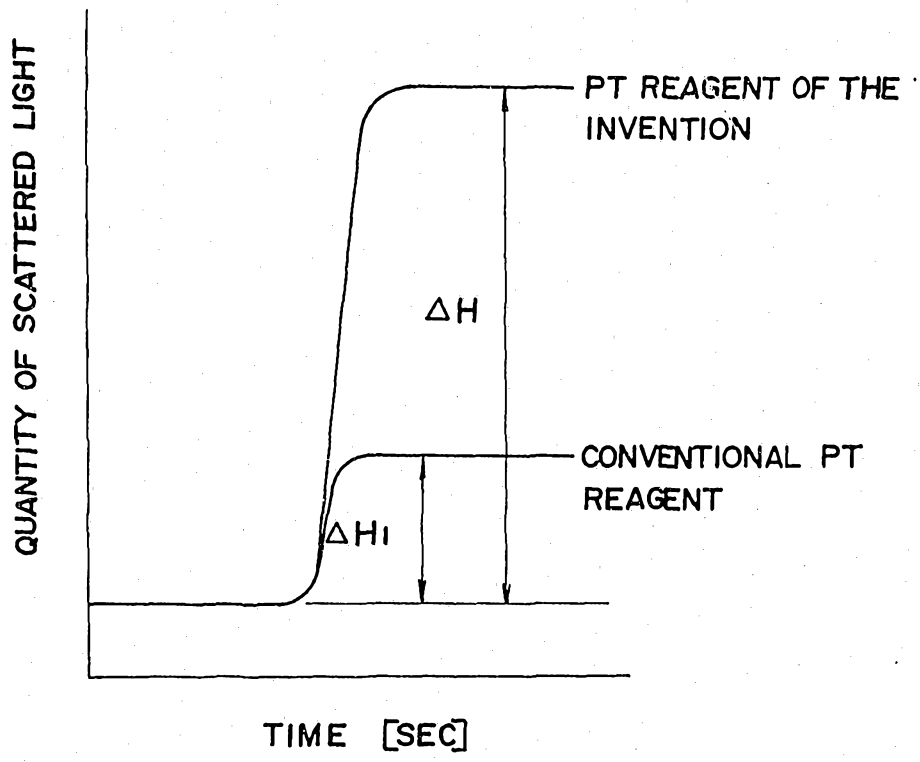

QUANTITY OF SCATTERED LIGHT
PT REAGENT OF THE INVENTION
ΔH
CONVENTIONAL PT REAGENT
ΔH1
TIME [SEC]

FIG.4

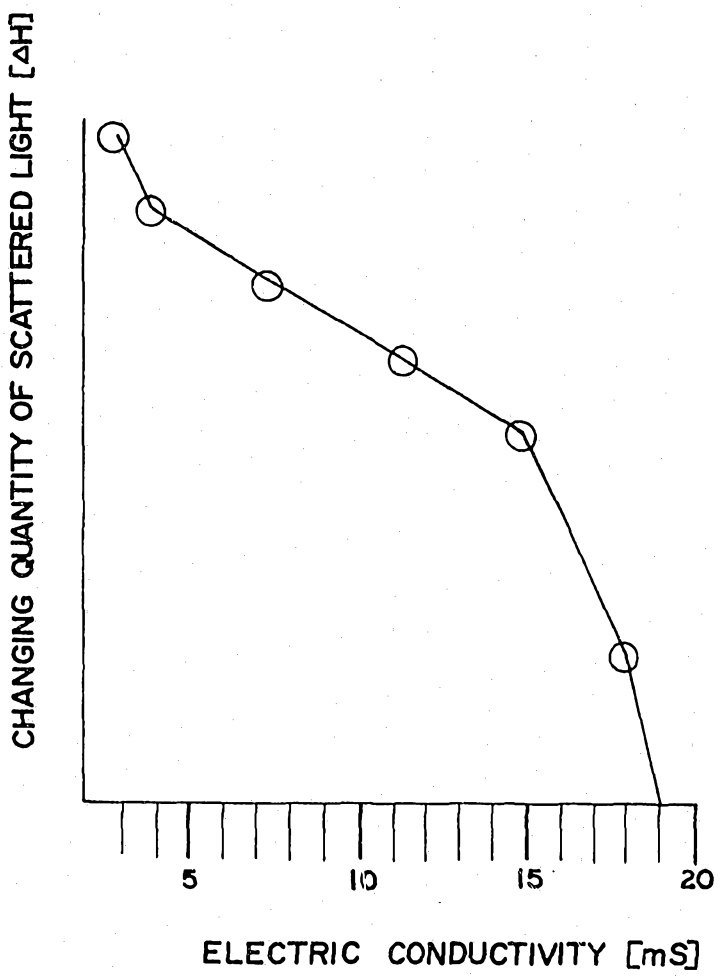

CHANGING QUANTITY OF SCATTERED LIGHT [ΔH]
5
10
15
20
ELECTRIC CONDUCTIVITY [mS]

FIG.5

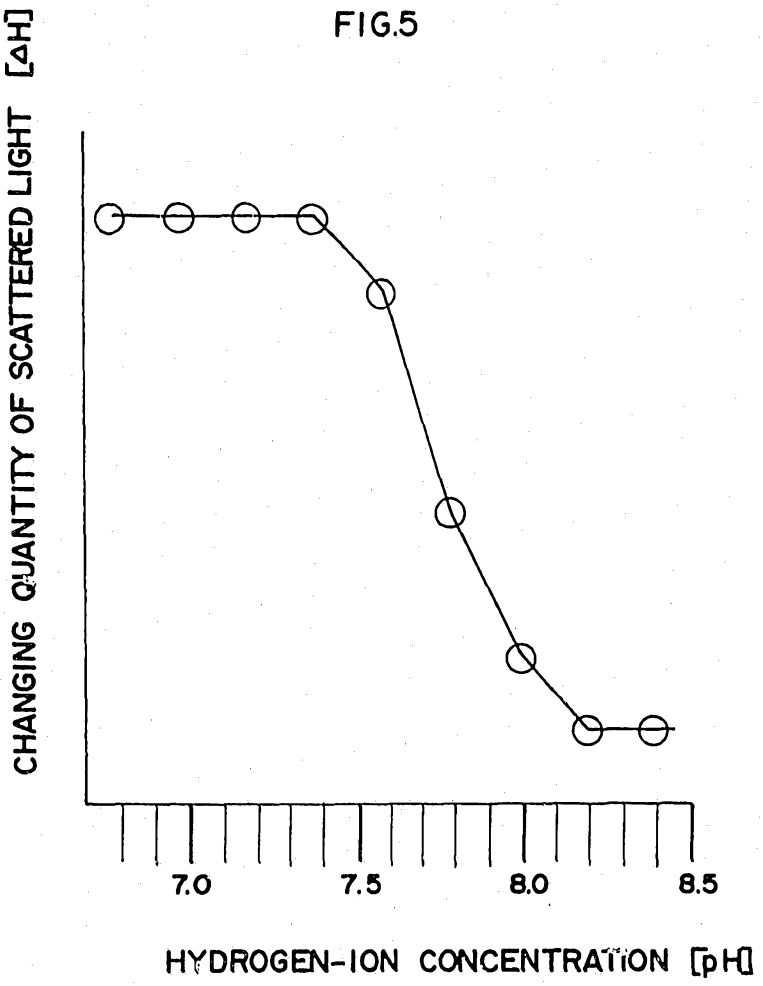
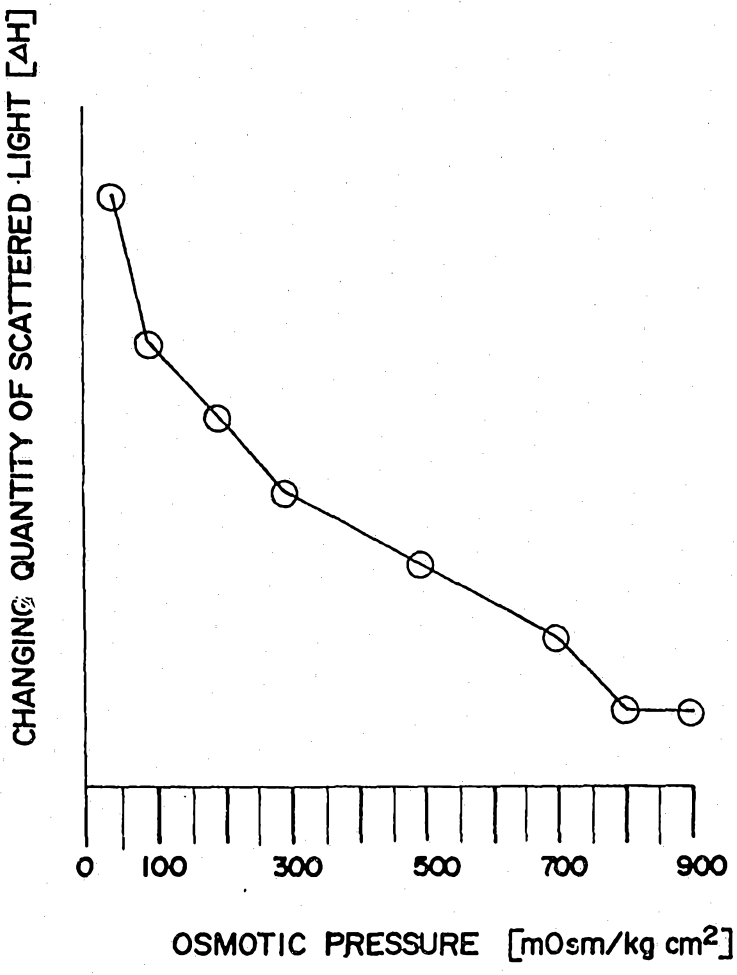

CHANGING QUANTITY OF SCATTERED LIGHT [ΔH]
7.0
7.5
8.0
8.5
HYDROGEN-ION CONCENTRATION [pH]

FIG.6


CHANGING QUANTITY OF SCATTERED LIGHT [ΔH]
0
100
300
500
700
900
OSMOTIC PRESSURE [mOsm/kg cm²]