polymer can be increased or decreased or the pattern of the distribution can be changed so that a high molecular weight redox polymer (composition) having the desired properties can be produced.

[0075]

Typically, the phenothiazine-based compound bound to a polymer according to the present invention for use as a redox polymer preferably has hydrophilicity. The details of the "hydrophilicity" are as mentioned above in relation to the hydrophilic moiety. For the phenothiazine-based compound bound to a polymer, it is preferred that the hydrophilic moiety or the high molecular weight polymer, or both, impart hydrophilicity to the phenothiazine-based compound bound to a polymer. For the phenothiazine-based compound bound to a polymer according to the present invention, it is preferred that the high molecular weight polymer be bound to the phenothiazine-based compound, for example, via a hydrophilic moiety (as one example, a PEG chain or other linker-like hydrophilic moieties), and it is more preferred that the high molecular weight polymer itself also have hydrophilicity.

[0076]

<High molecular weight polymer>

The high molecular weight polymer is a polymer having a given level or higher of a molecular weight (weight-average molecular weight) and may have a

may be measured by a known approach depending on the type of the high molecular weight polymer. For example, gel permeation chromatography (GPC) or SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (if the high molecular weight polymer is a protein) can be used. When a commercially available product is purchased and used as the high molecular weight polymer, a numeric value shown (e.g., as "Mw" or "M.W.") in its catalog or the like can be regarded as the weight-average molecular weight.

[0079]

The backbone of the high molecular weight polymer is generally a chain structure that is composed mainly of carbon atoms and may contain at least one heteroatom selected from the group consisting of a nitrogen atom, an oxygen atom, and a sulfur atom, in other words, a chain structure that may contain a heteroatom-containing bond such as an ether bond, a thioether bond, or an amide bond. The high molecular weight polymer appropriately has, at a side chain, a specific reactive group for binding to the phenothiazine-based compound (having a hydrophilic moiety introduced therein) and may further have a specific reactive group at one or both of the ends of the backbone, from the viewpoint that one molecule of the high molecular weight polymer can support a plurality of (many) phenothiazine-based compounds (having a hydrophilic moiety introduced therein). Various polymers are known as the high molecular weight polymer having

a formyl group (aldehyde group), an epoxy group, and a maleimide group". A protective group may be bonded, if necessary, to the specific reactive group of the high molecular weight polymer and may be deprotected before reaction for the binding of the high molecular weight polymer to the phenothiazine-based compound (having a hydrophilic moiety introduced therein).

[0081]

The specific reactive group of the high molecular weight polymer may be a group originally carried by a monomer used in the synthesis of the high molecular weight polymer or may be introduced later, for example, may be added by further introducing a reactive substituent to a reactive group originally carried by a monomer for the synthesis of the high molecular weight polymer. In the latter embodiment, the "reactive substituent" is the same as the group containing at least one of (a) to (m), which may be carried by the substituents (iii) to (viii), mentioned above in relation to the phenothiazine-based compound. In both the embodiments described above, the high molecular weight polymer can be regarded as "having" the specific reactive group.

[0082]

Specific examples of the high molecular weight polymer include amino acid-based polymers, imine-based polymers, and ethylene-based polymers having the specific

containing an appropriate specific reactive group therefor (e.g., a carboxy group or an amino group).

[0088]

In a preferred embodiment of the phenothiazine-based compound bound to a polymer according to the present invention, the high molecular weight polymer is a "cationic polymer". The "cationic polymer" is a generic name for polymers having a positive charge as a net of the whole polymer among the high molecular weight polymers as mentioned above. In general, the cationic polymer is a high molecular weight polymer having a positively charged functional group at a side chain, i.e., a high molecular weight polymer synthesized using a monomer having a positively charged functional group at a site that is not involved in backbone formation. Examples of the positively charged functional group include an amino group and a quaternary ammonium cation. A quaternary ammonium cation having chemical stability is preferred. Examples of the cationic polymer include homocopolymers or copolymers comprising a constituent unit derived from 2-aminoethyl methacrylate, (vinylbenzyl)trimethylammonium chloride, or methacryloyl choline chloride, which is disclosed as polymer A, B, or C of Examples, poly(diallyldimethylammonium chloride), poly(allylamine hydrochloride), allylamine hydrochloride-diallylamine hydrochloride copolymers, allylamine-diallyldimethylammonium chloride copolymers, and

metal or carbon material having conductivity and stability (e.g., oxidation resistance or salt tolerance). Typical examples of such a material of the electrode 22 include gold. Other examples thereof include platinum, palladium, and carbon. In the case of forming the working electrode 22a (the reagent layer 23) on one of the upper face and the lower face of the sensor 11 and forming the counter electrode 22c on the other face, electrode materials different from each other may be used.

[0114]

The working electrode 22a is given a potential (potential based on the reference electrode 22b) sufficient for oxidizing a mediator reduced through the reaction of an analyte (e.g., glucose) with an oxidoreductase. Glucose concentrations are measured by monitoring a current flowing between the working electrode 22a and the counter electrode 22c. The reagent layer 23 is formed on the upper face of the working electrode 22a in the tip portion of the sensor 11.

[0115]

The silver/silver chloride layer 24 is formed, if necessary, on the upper face of the reference electrode 22b in the tip portion of the sensor 11. In the present embodiment, an exemplary three-electrode configuration consisting of the working electrode 22a, the reference electrode 22b, and the counter electrode 22c is shown so

as to achieve more accurate measurement. A two-electrode configuration excluding the reference electrode 22b and consisting of the working electrode 22a and the counter electrode 22c is also possible (e.g., such a two-electrode configuration is mainstream for currently commercially available SMBG). The reference electrode can be prepared, as shown in the present embodiment, as a silver/silver chloride electrode provided with the silver/silver chloride (Ag/AgCl) layer 24. In addition, a mercury-containing layer can be formed, as in a hydrogen electrode, a calomel electrode, or the like.

[0116]

The film 25 is a sheet-shaped insulating member formed (laminated) on a predetermined portion of the electrode 22 (the working electrode 22a, the reference electrode 22b, and the counter electrode 22c) formed on the substrate 21, and the silver/silver chloride layer 24. The thickness of the film 25 is usually 1 μm or larger and 150 μm or smaller, preferably 3 μm or larger and 50 μm or smaller, more preferably 5 μm or larger and 30 μm or smaller. The film 25 has openings in a portion corresponding to the reagent layer 23 located at the tip of the sensor 11, and a portion corresponding to a portion of the counter electrode 22c, and the reagent layer 23 and the counter electrode 22c in the portions are exposed.

[0117]

a reference electrode region 112b through electric isolation by forming a groove 113 having a depth that reaches the surface of the insulating substrate 111 by laser lithography.

[0134]

The upper face of the insulating substrate 111 is coated with an insulating resist film 116a having an opening for forming a reference electrode 115 at a predetermined position of the reference electrode region 112b. The lower face of the insulating substrate 111 is coated with an insulating resist film 116b. On the other hand, neither the upper face nor the lower face is coated with the insulating resist film 116a or 116b from the end of the conductive thin film 112 to a given distant location. A region 112a on the front side serves as a working electrode 114, and a region 112c on the back side serves as a counter electrode 117. A reagent layer 118 is formed on the working electrode 114.

[0135]

Figure 5 shows a sectional view taken along the B-B' section line in Figure 4. Figure 6 shows a sectional view taken along the C-C' section line in Figure 4. As shown in Figure 5, at the B-B' section line, a protective film 119, a reagent layer 118, a working electrode 114, an insulating substrate 111, a counter electrode 117, and a protective film 119 are formed in order from the upper face toward the lower face (in the direction of the arrow

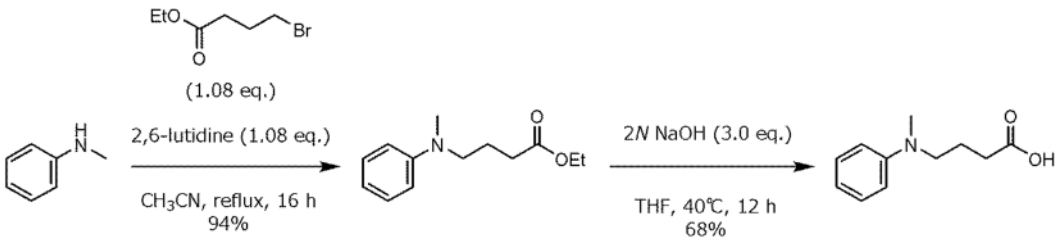
Figure 7(A) shows an electrode pattern before formation of a film (insulating resist film) 230. Figure 7(B) shows an electrode pattern after formation of the film 230.

[0137]

As shown in Figure 7(A), in the sensor 201, an electrode 220 is formed on a substrate 210. The electrode 220 is separated physically and electrically into a first working electrode 221, a second working electrode 222, a reference electrode 223, and a counter electrode 224 through a groove 225. As shown in Figure 7(B), the first working electrode 221 is coated with a film 230 and thereby has an exposed region 221a in the sensing portion 202 and an exposed region 221b in the terminal portion 203. Likewise, the second working electrode 222, the reference electrode 223, and the counter electrode 224 also respectively have exposed regions 222a, 223a, and 224a in the sensing portion 202 and exposed regions 222b, 223b, and 224b in the terminal portion 203. In the sensing portion 202, the exposed region 221a of the first working electrode and the exposed region 222a of the second working electrode are disposed side by side so as to maximize an area, while the exposed region 223a of the reference electrode and the exposed region 224a of the counter electrode are also disposed. Different types of reagent layers (not shown) for measuring different analytes can be formed on the

(2) Synthesis of carboxylic acid fragment

[Formula 17]

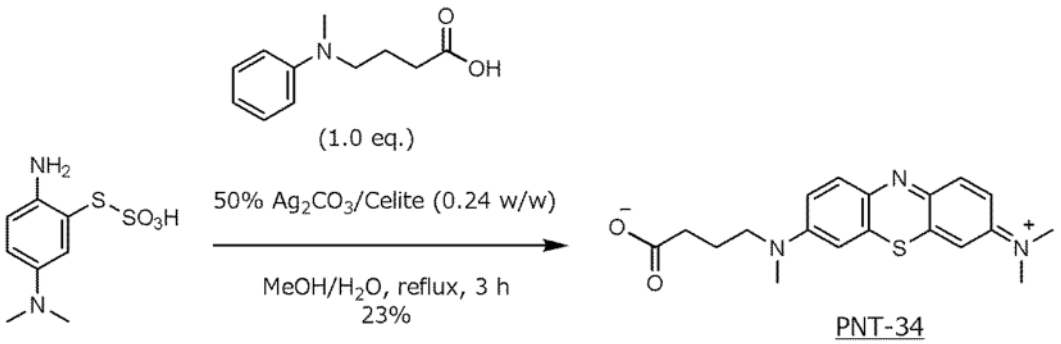


A carboxylic acid fragment was synthesized through the reaction described above.

[0154]

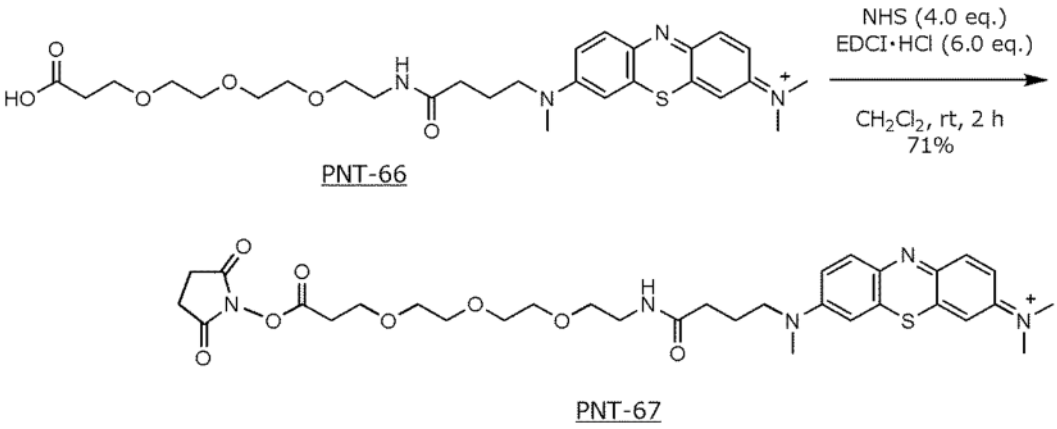
(3) Synthesis of PNT-34

[Formula 18]



The sulfonic acid fragment and the carboxylic acid fragment synthesized as described above were suspended in $MeOH/H_2O$, and 50% Ag_2CO_3/celite was added thereto in divided portions over 15 minutes at an internal temperature around 47°C. After addition, the mixture was stirred at an internal temperature around 68°C for 2.5 hours. The reaction mixture was allowed to cool to room temperature and filtered through celite, and the filtrate was concentrated. The residue was purified a plurality

[Formula 26]



PNT-66

PNT-67

A deprotected form (PNT-66) was dissolved in dichloromethane. To the solution, N-hydroxysuccinimide (NHS) and EDCI·HCl were added, and the mixture was then stirred at room temperature for 2 hours. The reaction solution was purified twice by silica gel column chromatography to obtain a PNT-66NHS form having an activated (NHS esterified) carboxy group at the end of a PEG chain (PEG3) (PNT-67).

[0165]

(2) Binding to poly(L-lysine)

The PNT-66NHS form (PNT-67) obtained in (1) was dissolved at 15 mg/mL in Milli-Q water (liquid (1)). Poly(L-lysine) hydrochloride (ALAMANDA POLYMERS PLKC800) was dissolved as a high molecular weight polymer at 10 mg/mL in Milli-Q water (liquid (2)). Next, WSC (DOJINDO W001) was dissolved at 20 mg/mL in Milli-Q water (liquid (3)). 43.7 μL of the liquid (1), 51.3 μL of the liquid (2), 479.3 μL of the liquid (3), and 96 μL of a 250 mM 2-morpholinoethanesulfonic acid (MES) buffer solution (pH

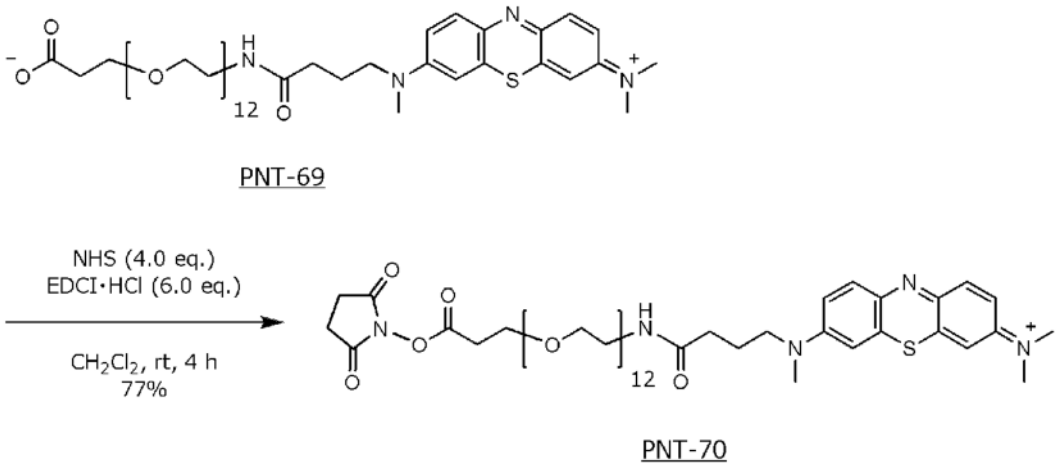
6.0) prepared separately were mixed and adjusted to a total volume of 1200 μL with Milli-Q water. Then, the mixture was reacted with stirring at room temperature for approximately 20 hours. Then, ultrafiltration was performed several times using a centrifugal ultrafiltration filter (Amicon Ultra-4 50k; Merck Millipore) to recover a liquid free from small molecules.

[0166]

[Example 3-2] Preparation of PNT-69 bound to polymer A

(1) Synthesis of PNT-69NHS form (PNT-70)

[Formula 27]



A deprotected form (PNT-69) was dissolved in dichloromethane. To the solution, N-hydroxysuccinimide (NHS) and EDCI·HCl were added, and the mixture was then stirred at room temperature for 4 hours. The reaction solution was purified by silica gel column chromatography to obtain a PNT-69NHS form having an activated (NHS esterified) carboxy group at the end of a PEG chain (PEG12) (PNT-70).

[0167]

(2) Binding to polymer A

A PNT-69NHS form (PNT-70) was dissolved at 21.05 mg/mL in Milli-Q water (liquid (1)). Polymer A was dissolved as a high molecular weight polymer at 15 mg/mL in Milli-Q water (liquid (2)). Next, WSC (DOJINDO W001) was dissolved at 20 mg/mL in Milli-Q water (liquid (3)). 50 μL of the liquid (1), 104.1 μL of the liquid (2), 479.3 μL of the liquid (3), and 96 μL of a 250 mM 2-morpholinoethanesulfonic acid (MES) buffer solution (pH 6.0) prepared separately were mixed and adjusted to a total volume of 1200 μL with Milli-Q water. Then, the mixture was reacted with stirring at room temperature for approximately 20 hours. Then, ultrafiltration was performed several times using a centrifugal ultrafiltration filter (Amicon Ultra-4 50k; Merck Millipore) to recover a liquid free from small molecules.

[0168]

[Example 3-3] Preparation of PNT-69 bound to polymer B

A PNT-69NHS form (PNT-70) was dissolved at 21.05 mg/mL in Milli-Q water (liquid (1)). Polymer B was dissolved as a high molecular weight polymer at 15 mg/mL in Milli-Q water (liquid (2)). Next, WSC (DOJINDO W001) was dissolved at 20 mg/mL in Milli-Q water (liquid (3)). 50 μL of the liquid (1), 405.2 μL of the liquid (2), 479.3 μL of the liquid (3), and 96 μL of a 250 mM 2-morpholinoethanesulfonic acid (MES) buffer solution (pH

6.0) prepared separately were mixed and adjusted to a total volume of 1200 μL with Milli-Q water. Then, the mixture was reacted with stirring at room temperature for approximately 20 hours. Then, ultrafiltration was performed several times using a centrifugal ultrafiltration filter (Amicon Ultra-4 50k; Merck Millipore) to recover a liquid free from small molecules.
[0169]
[Example 3-4] Preparation of PNT-69 bound to polymer C

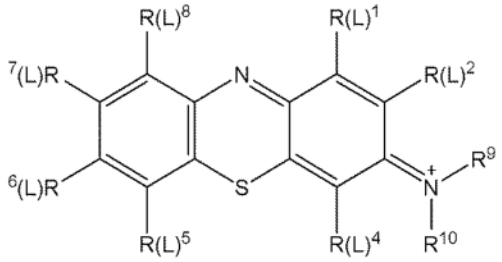
A PNT-69NHS form (PNT-70) was dissolved at 21.05 mg/mL in Milli-Q water (liquid (1)). Polymer C was dissolved as a high molecular weight polymer at 15 mg/mL in Milli-Q water (liquid (2)). Next, WSC (DOJINDO W001) was dissolved at 20 mg/mL in Milli-Q water (liquid (3)). 40 μL of the liquid (1), 140.3 μL of the liquid (2), 383.4 μL of the liquid (3), and 96 μL of a 250 mM 2-morpholinoethanesulfonic acid (MES) buffer solution (pH 6.0) prepared separately were mixed and adjusted to a total volume of 1200 μL with Milli-Q water. Then, the mixture was reacted with stirring at room temperature for approximately 20 hours. Then, ultrafiltration was performed several times using a centrifugal ultrafiltration filter (Amicon Ultra-4 50k; Merck Millipore) to recover a liquid free from small molecules.
[0170]
<Optical test>

CLAIMS

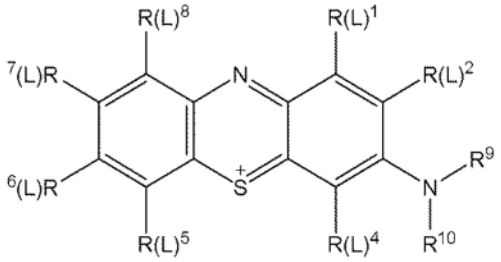
[Claim 1]

A phenothiazine-based compound having a hydrophilic moiety introduced therein, the compound being represented by the general formula (2A) or (2B):

[Formula 1]



(2A)



(2B)

wherein

R^9 and R^{10} are both substituents, or one of these moieties is a substituent, and the other moiety is a hydrogen atom,

$R(L)^1$, $R(L)^2$, and $R(L)^4$ to $R(L)^8$ are each independently [A] a hydroxy group optionally having a substituent, a carboxy group optionally having a substituent, an amino group optionally having a substituent, a linear or branched saturated or unsaturated hydrocarbon group having 1 to 6 carbon atoms and optionally having a substituent, an acyl group optionally having a substituent, or a phenyl group optionally having a substituent, [B] a group formed by the binding of a compound for hydrophilic moiety

introduction to the binding substituent selected from [A], or [C] a hydrogen atom or a halogen atom,

on the proviso that at least one of $R(L)^1$, $R(L)^2$, and $R(L)^4$ to $R(L)^8$, preferably, at least $R(L)^6$, is the group [B].

[Claim 2]

The phenothiazine-based compound having a hydrophilic moiety introduced therein according to claim 1, wherein the substituent represented by each of R^9 and R^{10} is an alkyl group having 1 to 6 carbon atoms.

[Claim 3]

The phenothiazine-based compound having a hydrophilic moiety introduced therein according to claim 1 or 2, wherein at least one of $R(L)^1$, $R(L)^2$, and $R(L)^4$ to $R(L)^8$, preferably, at least $R(L)^6$, is a group formed by the binding of a compound for hydrophilic moiety introduction to:

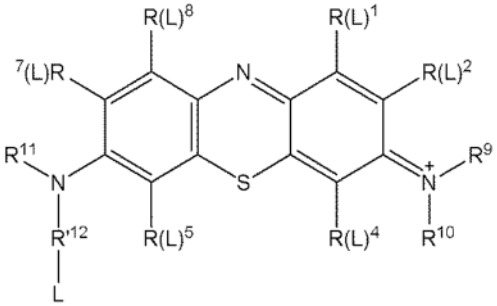
a binding substituent having at least one reactive group selected from the group consisting of a carboxy group or an active ester form thereof, an amino group, a thiol group, a formyl group, an epoxy group, and a maleimide group; or

a binding substituent which is an amino group having a substituent, the binding substituent being represented by $-NR^{11}R^{12}$ (wherein one of R^{11} and R^{12} is a reactive N substituent, and the other moiety is a hydrogen atom or a nonreactive N substituent).

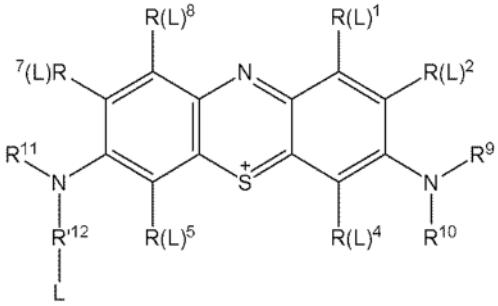
[Claim 4]

The phenothiazine-based compound having a hydrophilic moiety introduced therein according to claim 1, wherein the compound is represented by the general formula (2A-2) or (2B-2):

[Formula 2]



(2A-2)



(2B-2)

wherein R^{11} is a hydrogen atom or a nonreactive N substituent, R'^{12} is a group derived from a reactive N substituent, L is the hydrophilic moiety derived from the compound for hydrophilic moiety introduction, and the other symbols are as defined in the general formulas (2A) and (2B).

[Claim 5]

The phenothiazine-based compound having a hydrophilic moiety introduced therein according to any one of claims 1 to 4,

wherein the hydrophilic moiety derived from the compound for hydrophilic moiety introduction comprises at least one member selected from the group consisting of a hydroxy group, an amino group, a carboxy group, a sulfo

group, a quaternary ammonium cation, and groups derived therefrom; and/or

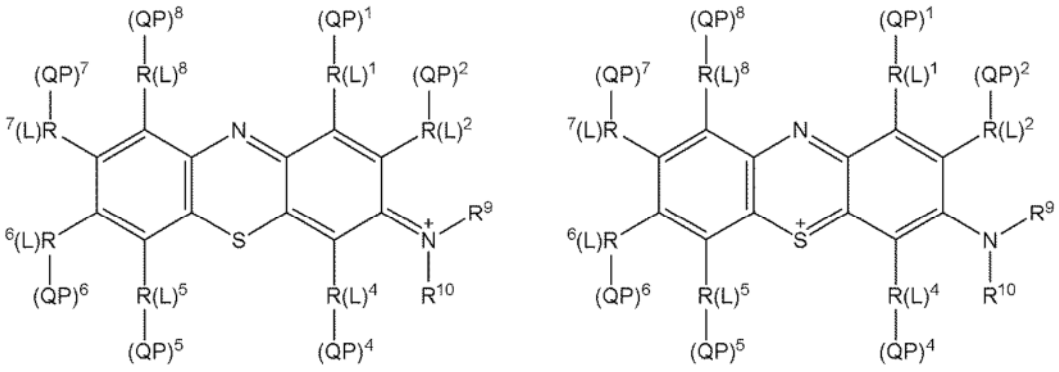
wherein the hydrophilic moiety derived from the compound for hydrophilic moiety introduction comprises an oxyethylene chain; and/or

wherein the hydrophilic moiety derived from the compound for hydrophilic moiety introduction has at least one reactive group selected from the group consisting of a carboxy group or an active ester form thereof, an amino group, a thiol group, a formyl group, an epoxy group, and a maleimide group.

[Claim 6]

A phenothiazine-based compound bound to a polymer, the compound being represented by the general formula (3A) or (3B):

[Formula 3]



(3A) (3B)

wherein

R^9 and R^{10} are both substituents, or one of these moieties is a substituent, and the other moiety is a hydrogen atom,

$R(L)^1$, $R(L)^2$, and $R(L)^4$ to $R(L)^8$ are each independently [A] a hydroxy group optionally having a substituent, a carboxy group optionally having a substituent, an amino group optionally having a substituent, a linear or branched saturated or unsaturated hydrocarbon group having 1 to 6 carbon atoms and optionally having a substituent, an acyl group optionally having a substituent, or a phenyl group optionally having a substituent, [B] a group formed by the binding of a compound for hydrophilic moiety introduction to the binding substituent selected from [A], [C] a hydrogen atom or a halogen atom, or [D] a group derived from either the binding substituent selected from [A] or the group [B],

preferably, on the proviso that at least $R(L)^6$ is a group derived from either the binding substituent selected from [A] or a group formed by the binding of a compound for hydrophilic moiety introduction to the binding substituent, preferably the binding substituent selected from [A].

$(QP)^1$, $(QP)^2$, and $(QP)^4$ to $(QP)^8$ are each independently a polymeric structure derived from a high molecular weight polymer which is present when their corresponding $R(L)^1$, $R(L)^2$, and $R(L)^4$ to $R(L)^8$ are the group [D], and

on the proviso that at least one of $(QP)^1$, $(QP)^2$, and $(QP)^4$ to $(QP)^8$ is present.

[Claim 7]

The phenothiazine-based compound bound to a polymer according to claim 6, wherein the substituent represented by each of R^9 and R^{10} is an alkyl group having 1 to 6 carbon atoms.

[Claim 8]

The phenothiazine-based compound bound to a polymer according to claim 6 or 7, wherein at least one of $R(L)^1$, $R(L)^2$, and $R(L)^4$ to $R(L)^8$, preferably, at least $R(L)^6$, is a group derived from either:

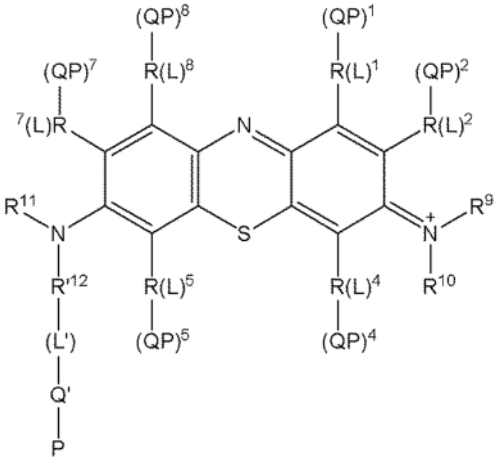
a binding substituent having at least one reactive group selected from the group consisting of a carboxy group or an active ester form thereof, an amino group, a thiol group, a formyl group, an epoxy group, and a maleimide group, or a binding substituent which is an amino group having a substituent, the binding substituent being represented by $-NR^{11}R^{12}$ (wherein one of R^{11} and R^{12} is a reactive N substituent, and the other moiety is a hydrogen atom or a nonreactive N substituent); or

a group formed by the binding of a compound for hydrophilic moiety introduction to the binding substituent.

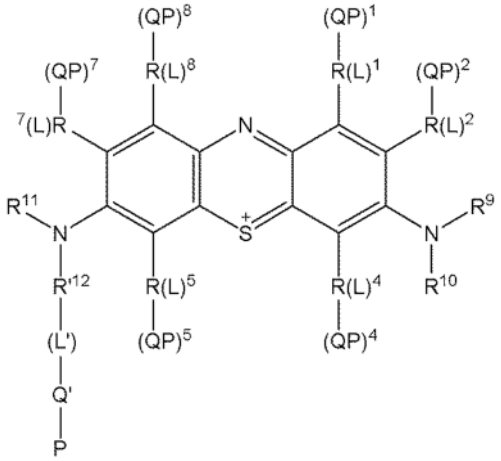
[Claim 9]

The phenothiazine-based compound bound to a polymer according to claim 6, wherein the compound is represented by the general formula (3A-2) or (3B-2):

[Formula 4]



(3A-2)



(3B-2)

wherein R^{11} is a hydrogen atom or a nonreactive N substituent, R'^{12} is a group derived from a reactive N substituent, (L') is optionally present and is a group derived from a compound for hydrophilic moiety introduction, Q' is a group derived from a reactive group of a high molecular weight polymer, P is a backbone of the high molecular weight polymer, and the other symbols are as defined in the general formulas (3A) and (3B).

[Claim 10]

The phenothiazine-based compound bound to a polymer according to any one of claims 6 to 9,

wherein the hydrophilic moiety derived from the compound for hydrophilic moiety introduction comprises at least one member selected from the group consisting of a hydroxy group, an amino group, a carboxy group, a sulfo group, a quaternary ammonium cation, and groups derived therefrom; and/or

wherein the hydrophilic moiety derived from the compound for hydrophilic moiety introduction comprises an oxyethylene chain.

[Claim 11]

The phenothiazine-based compound bound to a polymer according to any one of claims 6 to 10, wherein at least one of $R(L)^1$, $R(L)^2$, and $R(L)^4$ to $R(L)^8$ is a group derived from a group formed by the binding of a compound for hydrophilic moiety introduction having at least one reactive group selected from the group consisting of a carboxy group or an active ester form thereof, an amino group, a thiol group, a formyl group, an epoxy group, and a maleimide group to the binding substituent.

[Claim 12]

The phenothiazine-based compound bound to a polymer according to any one of claims 6 to 11, wherein the high molecular weight polymer is:

at least one member selected from the group consisting of an amino acid-based polymer, an imine-based polymer, an ethylene-based polymer, and a protein; or

a cationic high molecular weight polymer.

[Claim 13]

A reagent layer comprising a phenothiazine-based compound having a hydrophilic moiety introduced therein according to any one of claims 1 to 5, or a phenothiazine-based compound bound to a polymer according to any one of claims 6 to 12.

[Claim 14]

The reagent layer according to claim 13, further comprising an oxidoreductase that oxidizes or dehydrogenates an analyte, preferably,

wherein the oxidoreductase is cross-linked to the phenothiazine-based compound bound to a polymer.

[Claim 15]

An electrochemical sensor for detecting or quantifying an analyte, the electrochemical sensor having a working electrode, a counter electrode, and the reagent layer according to claim 13 or 14 disposed on the working electrode; preferably,

further having a reference electrode; preferably,

further having a protective film with which at least the reagent layer is coated.