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(54) Title: WATER-SOLUBLE CONJUGATES AND METHODS OF PREPARATION

(57) Abstract: The present invention provides water-soluble conjugates and methods of using them in diagnostic and detection assays. Devices for performing detection and quantitation assays are also provided. In various embodiments the conjugates are useful in immunoassays and later flow assays. The invention provides methods of preparing the conjugates that result in higher yields and higher sensitivities for the assays.

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WATER-SOLUBLE CONJUGATES AND METHODS OF PREPARATION

Field of the Invention

[0001] The present invention relates to compositions of water-soluble conjugates useful in diagnostic assays. The invention also relates to methods for preparing water-soluble conjugates, immunoassays, lateral flow assays, and test devices.

Background of the Invention

[0002] There is a continuing need for superior methods of preparing conjugates which exhibit a high degree of sensitivity and specificity when employed in immunochemical assays, such as home pregnancy and fertility tests.

[0003] Various strategies for improving the sensitivity and reliability of immunoassays have been reviewed by L. J. Kricka (1994) Clin. Chem. 40, 347-357.

[0004] EP 0 594 772 B1 relates to water-soluble, polymer-based conjugates comprising moieties derived from divinyl sulfone. EP 0 594 772 B1 describes the possibility of enhancing the attachment of molecular species, such as antibodies and antigens, to a water-soluble carrier molecule by taking advantage of the so-called "salting out" effect. It turned out, however, that by increasing the salt concentration to about 1 M an irreversible precipitate was formed.

[0005] U.S. Patent No. 6,627,460 to Lihme et al., provides methods of water-soluble cross linked conjugates, and methods of their use. The patent provides methods of further increasing the concentration of salt in the reaction mixture, which causes a reversible (i.e. a re-dissolvable) precipitate to form containing a water-soluble conjugate, which is useful in various immunochemical assays such as in lateral flow devices.

Summary of the Invention

[0006] The present invention provides compositions of water-soluble conjugates for use in diagnostic and detection assays, and methods of their preparation. In various embodiments the conjugates are useful in immunoassays and lateral flow assays. The invention provides methods of preparing the conjugates that result in higher yields in the preparation of the conjugates, and higher sensitivities in

the assays. The invention also provides devices for use in conducting detection and quantitation assays for a variety of ligands of interest.

[0007] In a first aspect the invention provides methods for preparing a water-soluble conjugate involving a) preparing a water-soluble conjugate having at least one carrier, at least one linker, at least one signal component, and at least one targeting element for a ligand to be detected or a ligand to be detected, as a reversible precipitate in a suspension. The suspension is subjected to sonication to form a sonicated formulation, and a supernatant is separated from the formulation containing the water-soluble conjugate. Optionally, the water-soluble conjugate can be purified from the supernatant.

[0008] In various embodiments the water-soluble conjugate can also contain a spacer component. In one embodiment the carrier is covalently attached to the linker and the signal component is covalently attached to the spacer. The water-soluble conjugate can be prepared by contacting a water-soluble intermediate conjugate with the ligand to be detected or the targeting element for a ligand in the presence of a lyotropic salt at a concentration of at least about 1.25 M. A "water-soluble conjugate" contains a carrier, a linker, a targeting element for a ligand to be detected or a ligand to be detected, and can optionally also contain a spacer component. By "water-soluble intermediate conjugate" is meant a molecule containing a carrier, a linker, and a signal component. A water-soluble intermediate conjugate may also contain a spacer component. By "water-soluble intermediate precursor" is meant a molecule having any two or more of the components of a water-soluble conjugate and that is not a water-soluble conjugate. In one embodiment the water-soluble intermediate conjugate contains the carrier, linker, signal component, and spacer components. "Sonication" refers to the known technique used in chemistry and biology of exposure to a high frequency sound energy. It is also sometimes referred to as ultrasonication. The sonication can performed at any appropriate power, e.g., at least about 300 watts, or at least about 500 watts, or at least about 700 watts, or at least about 900 watts, or at least about 1000 watts, or at greater than 1000 watts. Any desirable frequency can also be used, such as from 20 to 24 kHz. As used herein, "about" means plus or minus 10%.

[0009] The lyotropic salt can contain components such as sulphates, phosphates, citrates and tartrates of lithium, sodium, potassium, calcium and

ammonium, and can be present at a concentration of about 2.5 M. In one embodiment the salt is potassium phosphate or sodium phosphate.

[0010] In one embodiment the water-soluble conjugate is separated from the supernatant by centrifugation, although centrifugation is not necessary to practicing the method. The conjugate can also be purified from the supernatant by any convenient techniques, such as by gel filtration. By "supernatant" is meant the liquid portion of a sample.

[0011] Methods of preparing water-soluble conjugates are discussed in U.S. Patent 6,627,460, which is hereby incorporated by reference in its entirety, including all tables, figures, and claims. These methods generally involve the preparation of a water-soluble cross-linked conjugate having a carrier component, a linking component, a spacer component, a signal component and a targeting element for a ligand to be detected or a ligand to be detected (a primary targeting component). The signal component is covalently attached to the spacer component and the spacer component is covalently attached, via the linking component, to the carrier component. The methods involve a) reacting a water-soluble intermediate conjugate having a carrier component, a linking component, a spacer component, and a signal component (the signal component being covalently attached to the spacer component and the spacer component being covalently attached, via the linking component, to the carrier component), with at least one primary targeting component (a targeting element for a ligand to be detected or ligand to be detected). The reaction occurs with unreacted reactive moieties derived from the linking component, in an aqueous solution. The conditions are such that a reversible precipitate is formed. The reversible precipitate containing the water-soluble conjugate is re-dissolved in an aqueous medium; and c) optionally, the water-soluble cross-linked conjugate is subjected to a purification step. Further details of the reaction parameters are provided in U.S. Patent No. 6,627,460, which is hereby incorporated by reference in its entirety, including all tables, figures, and claims.

[0012] While examples of the arrangement of the water-soluble conjugates are provided herein, other arrangements are possible. For example, the targeting element can be attached to the carrier via the linker, or can be attached to the spacer or to a non-specific protein, as described below. Also, the signal component can be attached to the carrier, or to the spacer, or even to the targeting element. The precise arrangement of components can be varied in any manner to result in a water-soluble

conjugate that functions as a reagent and the assay is performed and provides a useful result.

[0013] In the present context the term "water soluble" when used in connection with the cross-linked conjugates means that the conjugates obtained should be soluble in an aqueous medium, such as water, at room temperature, i.e. the cross-linked conjugates obtained by the methods disclosed herein should give rise to a solution which is substantially clear and homogenous as judged by visual inspection of the sample.

[0014] In various embodiments the cross-linked conjugates obtained have a water solubility of at least 0.1, or at least 0.2 or at least 0.5, or at least 1, or at least 3, or at least 5, or at least 7, or from 5 to 10, or from 4 to 11, or at least 10, or at least 20, or at least 30, or at least 40, or at least 50, or at least 100, and in particular at least 200 mg dry conjugate per ml water at 25 °C.

The Carrier

[0015] The term "carrier" in the context of the present invention is used to denote the "backbone" of the conjugate, i.e. the carrier component functions as a backbone on which various components may be attached. The water-soluble polymers which function as the carrier component in the method for the preparation of conjugates may be chosen from a wide variety of types of polymers, including: natural and synthetic polysaccharides, as well as derivatives thereof, for example dextrans and dextran derivatives, starches and starch derivatives, cellulose derivatives, amylose and pectin, as well as certain natural gums and derivatives thereof, such as gum arabic and salts of alginic acid; homopoly(amino acid)s having suitable reactive functionalities, such as polylysines, polyhistidines or polyornithines; natural and synthetic polypeptides and proteins, such as bovine serum albumin and other mammalian albumins; and
synthetic polymers having nucleophilic functional groups, such as polyvinyl alcohols, polyallyl alcohol, polyethylene glycols and substituted polyacrylates.

[0016] Very suitable polymers for the purposes of the invention are polysaccharides and derivatives thereof, for example: dextrans, carboxymethyl-dextrans, hydroxyethyl- and hydroxypropyl-starches, glycogen, agarose derivatives, and hydroxyethyl- and hydroxypropyl-celluloses. As will be apparent from the

working examples herein (vide infra), notably dextrans have proved to be particularly suitable polymers in connection with the invention.

[0017] It is often desirable, particularly for many of the immunochemical applications of the conjugates, that the conjugates have no, or substantially no, net charge, since the presence of a net positive or negative charge in such cases can lead, *inter alia*, to undesirable non-specific binding of the conjugates to substances and/or materials other than those of interest. In many cases this condition will, unless charged species are introduced, be fulfilled simply by ensuring that the polymeric carrier component itself possesses no net charge. Thus, a suitable polymeric carrier component for use in the method of the invention is, in its free state, substantially linear and substantially uncharged at a pH in the range of about 4 to about 10, the latter pH interval being the interval of practical relevance for the vast majority of immunochemical procedures, hybridization procedures and other applications of conjugates. Among various polymers which meet this criterion, are, for example, numerous polysaccharides and polysaccharide derivatives, e.g. dextrans and hydroxyethyl- and hydroxypropylcelluloses.

[0018] Depending on the use to which a conjugate is to be put, the conjugates may be based on water-soluble polymeric carrier components having a range of molecular weights. In one embodiment of the invention, the polymeric carrier component may have a peak molecular weight in the range of about 40,000 to about 40,000,000 (prior to reacting the water-soluble polymeric carrier components with linker reagent such as DVS (divinyl sulfone) or EPCH (epichlorhydrin), or reacting resulting water-soluble intermediate precursor with a spacer or signal component for the eventual formation of cross-linked conjugate and cross-linked conjugate complexes). Peak molecular weights which are of considerable interest are peak molecular weights in the range of 100,000 to 10,000,000, such as in the range from 500,000 to 8,000,000, or in the range from 500,000 to 4,000,000, e.g. in the range from 500,000 to 2,000,000. Peak molecular weights of particular interest, notably in the case of dextrans as polymeric carrier components, are peak molecular weights of about 500,000, about 1,000,000, about 1,500,000, about 2,000,000, 2,500,000, about 3,000,000, about 3,500,000 and about 4,000,000.

[0019] More particularly, dextrans in the molecular weight ranges of 20,000 to 2,000,000 are suitable as starting carrier components. Most particularly, 20,000 Da dextrans are suitable for, but not restricted to, conjugates and/or complexes using

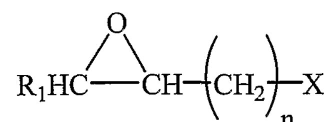
streptavidin as the primary or secondary target. Furthermore, 500,000 Da dextrans are suitable for, but not restricted to, conjugates and/or complexes using certain dyes, enzymes, and with certain specific binding molecules as the primary or secondary target. Moreover, 2,000,000 Da dextrans are suitable for, but not restricted to, certain other dyes. In different embodiments the carrier can be any suitable carrier molecule, such as, for example, dextran, starch, glycogen, agarose, cellulose, natural gum, or mixtures thereof.

[0020] The term "peak molecular weight" as employed in the present specification and claims in connection with the carrier components denotes the molecular weight of greatest abundance, i.e. that molecular weight, among a distribution of molecular weights, which is possessed by the greatest number of molecules in a given sample or batch of the polymer. It is quite normal to characterize numerous types of polymers in this manner, owing to the difficulty (particularly for the highest molecular weights) of obtaining or preparing polymer fractions of very narrow molecular weight distribution. In the case of numerous commercially available carrier components which are of interest in the context of the invention, for example dextrans, the manufacturer or distributor will be able to provide reliable peak molecular weight data (determined, for example, by gel-permeation chromatography) which can provide a basis for the selection of the proper fraction of the polymeric carrier component. It should be mentioned here that peak molecular weight values (when used in connection with the carrier component) cited in the present specification and claims refer to the peak molecular weight of the free polymer in question, and take no account of, for example, the possible formation of cross-linked polymer units, e.g. as a result of cross-linking of two or more polymer molecules by reaction with a linking component such as DVS or EPCH during a method for the preparation of a conjugate; such cross-linked units will, on average, have higher molecular weights than the individual free polymer molecules from which they are formed.

The Linker Component

[0021] In the present context the terms “linker” or “linking component" are intended to cover bi-functional molecules capable of establishing covalent links between other--typically larger--molecules. Examples of linking components suitable for the method according to the invention are e.g. molecules comprising a bi-

functional reactivity such as glutaraldehyde, carbodiimides, N,N'-phenylenedimaleimide, N-succinimidyl 3-(2-pyridylthio)propionate, p-benzoquinone, bis-oxiranes, divinyl sulfone (DVS) and epoxide derivatives, such as epoxides of the general formula I:



wherein R_1 is hydrogen or C_{1-4}-alkyl, n is an integer in the range from 1-4, i.e. 1, 2, 3 or 4, and X is a leaving group such as tosyl, mesyl, or halogen such as fluorine, chlorine, bromine, or iodine. In the present context the term "C_{1-4} -alkyl" designates a straight or branched saturated hydrocarbon group having from 1 to 4 carbon atoms, such as methyl ethyl, n-propyl, n-butyl, isopropyl, isobutyl, etc. As will be apparent from the working examples provided herein a very promising epoxide-derived linking component is epichlorohydrin (EPCH), i.e. a compound of the general formula I above, wherein R_1 is hydrogen, n is 1 and the leaving group X is chlorine.

[0022] The linking component should be stable in an aqueous environment and, accordingly, the linking component EPCH constitutes together with the linking component DVS a very useful linking components for use in the methods of the invention.

The Spacer Component

[0023] The spacer component is, via reaction with the linking component, covalently attached to the water-soluble intermediate precursor, thereby forming a second water-soluble intermediate precursor. As indicated above, the "spacer component" is covalently attached, via the linking group, to the carrier component. Thus, the term "spacer component" when used in the present context indicates a protein or a polypeptide which has a plurality of sites available for covalent attachment of signal components, such as dyes (vide infra). Spacer components are useful in any of the water-soluble conjugates used herein and in any of the methods of preparing water-soluble conjugates, although it is also possible for the conjugates to function without a spacer component.

[0024] One purpose for the incorporation of a spacer component, and particularly for a spacer having a plurality of sites available for covalent attachment of signal components, is that this method provides for a suitable means of increasing the number of signal components which can be attached to the conjugate (i.e. the "load"

of the signal component in the water-soluble intermediate conjugate, vide ante), and thereby increasing the sensitivity of such conjugates when employed in various assays, e.g. immunochemical assays and in the lateral flow devices described herein (vide infra). It should be understood that in an embodiment wherein the coupling of a signal component (such as a dye molecule) is done directly to the linking component (and not through a spacer component) implies that (at least in principle) only one signal molecule is attached per molecule of linking component present in the conjugate.

[0025] In several embodiments of the preparation of the second water-soluble precursor, the number of moles of spacer per mole of starting dextran (the "load" of the spacer) ranges from 1 to 500, particularly from 2 to 100, most frequently from 5 to 75. Also, the second water-soluble intermediate can be characterized by, e.g., the number (moles) of spacer component attached per mole carrier component.

[0026] As stated earlier, only a fraction of the reactive moieties of the linking component of the water-soluble intermediate reacts with the spacer component. Depending on the spacer component and on the linker component, after reacting the spacer component, from 1 to 99% of the unreacted reactive moieties of the linker component, or 20-99%, particularly 30-99%, such as ranging from 40 to 99% and notably 50 to 99% remain unreacted. That is to say that, in one embodiment, under certain conditions, from 1 to 49% of the unreacted linker moieties reacted with the spacer component.

[0027] The spacer component can be a protein such as BSA, ovalbumin, globulin, etc. or a polypeptide such as homopolypeptides, e.g. polylysines, polyhistidines, polyornithines, etc. However, the choice of spacer component will depend on the employed signal component (e.g. the actual dye employed in a particular conjugate) as well as the employed linking component.

[0028] The molecular weight of the spacer component, e.g. a protein, can be at least 2,500 Da, or at least 5,000 Da, or at least 10,000 Da, or in the range of 10,000-2,000,000, such as in the range of 20,000-500,000. As the one of the features of the introduced spacer components is to multiply the number of available positions for introduction of the signal components, it is furthermore desirable that the number of available functional groups for attachment of signal components is at least 5 per molecule of spacer component, for example, 10-1,000, in particular 10-500.

[0029] Alternatively, the spacer component can be a polysaccharide or polynucleic acid. Chemical modifications of these polymers may be required prior to the preparation of the water-soluble intermediate conjugate.

[0030] Owing to the nature of the coupling chemistry on the spacer component, (to both the linker component in the formation of the second water-soluble intermediate precursor, or later to a signal component in the formation of the water-soluble-intermediate conjugate, vide infra), a reactive functionality, such as a nucleophilic functionality, is present on the spacer component. Suitable spacer components will then be, for example, those with nucleophilic functional groups such as: --O^- (e.g. deprotonated phenolic hydroxy groups, such as deprotonated aromatic hydroxy groups in tyrosine residues of polypeptides or proteins), --S^- (e.g. deprotonated thiol groups on aromatic rings or aliphatic groups, such as deprotonated thiol groups in cysteine residues of polypeptides or proteins), --OH (e.g. aliphatic hydroxy groups present in certain amino acid residues of polypeptides or proteins, such as serine or threonine residues), --SH (e.g. thiol groups in cysteine residues of polypeptides or proteins), primary amino groups (e.g. in lysine or omithine residues of polypeptides or proteins) or secondary amino groups (e.g. in histidine residues of polypeptides or proteins). As will be understood by the skilled person, the question of whether the functional groups mentioned above will be in a protonated or de-protonated state will, of course, depend on the selected reaction conditions, such as the pH of the reaction mixture.

[0031] In one embodiment only a fraction of the unreacted reactive moieties of the linker component of the water-soluble intermediate react with the spacer component. That is to say that the second water-soluble intermediate still possesses a significant amount of unreacted reactive moieties.

[0032] The obtained second water-soluble intermediate precursor may be purified by the methods already discussed in connection with the purification step, i.e. in connection with the purification of the water-soluble intermediate precursor. As will be evident from the examples provided herein, a suitable method for purifying the obtained second water-soluble intermediate precursor is gel-filtration.

The Signal Component

[0033] The signal component is, via reaction with the spacer component, covalently attached to the second water-soluble intermediate precursor, thereby forming a water-soluble intermediate conjugate.

[0034] As used herein, the term "signal component" refers to moieties which are directly physically detectable or which are precursors for or produce such physically detectable moieties. In one embodiment the signal component functions as a label or a marker which can be readily measured by some physical technique known in art, e.g. by means of optical methods, such as spectrophotometry, fluorescence, luminescence, phosphorescence or other methods such as those described in e.g. "Instrumental Methods of Chemical Analysis" G. W. Ewing, 5th Ed., McGraw-Hill Book Company, New York, 1988. The signal component can also be detected by visual observation with the unaided eye. Alternatively, the signal component may--as indicated above--be a precursor for such a physically detectable component. A typical example of such a precursor is an enzyme which upon action on a suitable substrate is capable of generating species, for example colored species, which can be detected by one or more of the physical methods mentioned above.

[0035] The signal component may be selected from substances such as dyes; fluorescent, luminescent, phosphorescent and other light-emitting substances; metal-chelating substances, including iminodiacetic acid, ethylenediaminetetraacetic acid (EDTA), diethylene triaminepentaacetic acid (DTPA) and desferrioxamine B; substances labelled with a radioactive isotope; substances labelled with a heavy atom; and mixtures thereof.

[0036] To give some further examples, fluorescent substances may be selected from, e.g., fluorescein (suitably as fluorescein isothiocyanate, FITC), fluoresceinamine, 1-naphthol, 2-naphthol, eosin, erythrosin, morin, o-phenylenediamine, rhodamine and 8-anilino-1-naphthalenesulfonic acid. Radioactive isotopes of relevance may be selected, for example, among isotopes of hydrogen (i.e. tritium, ^{3}H), carbon (such as ^{14}C), phosphorus (such as ^{32}P), sulfur (such as ^{35}S), iodine (such as ^{131}I), bismuth (such as ^{212}Bi), yttrium (such as ^{90}Y), technetium (such as ^{99m}Tc), palladium (such as ^{109}Pd) and samarium (such as ^{153}Sm). Heavy atoms of relevance may be selected, for example, among Mn, Fe, Co, Ni, Cu, Zn, Ga, In, Ag, Au, Hg, I, Bi, Y, La, Ce, Eu and Gd. Gold (Au) is a particularly useful heavy atom in many cases.

[0037] Signal components which are considered of special interest are the non-particulate labels, for example, non-particulate dyes. In the present context the term "dye" is intended to mean any spectrophotometrically detectable dye molecule or derivative thereof. Dyes useful for incorporation into the conjugates prepared by the methods according to the invention include those derived from visual dyes, phosphorescent dyes, fluorescent dyes, laser dyes, infrared dyes and lanthanide chelates. Dyes which are particular interesting are visual dyes, including soluble visual dyes, such as pigments, vat dyes, sulphur dyes, mordant dyes, leucovat dyes and species such as fluorescein, rhodamine and derivatives thereof (such as sulphorhodamine, rhodamine-hydride and rhodamine hydrazide), as well as oxazine dyes, cyanine dyes and azol dyes. Specific examples of suitable dyes are, for example, Texas Red hydrazine, Congo Red, Trypan Blue, Lissamine Blue, Remazol Black, Remazol Brilliant Red, Rhodamine B Isothiocyanate, Cy5-Osu mono functional reactive dye, Reactive Orange 16, Uniblue A, etc. Dyes that are not particulate dyes are also useful in the present invention. A "non-particulate" label is one where the basis of detection of the label is other than the detection of a solid, whether the solid is the signal component (e.g., latex or other particles) or whether a solid precipitate is produced that is the basis for detection.

[0038] The above-mentioned dyes, which are useful as signal components for the purposes of the present invention, are all well-known in the art and it will be clear to the skilled person that other dyes can be used as signal components for the purposes of the present invention. Other examples of dyes to be used as signal components are e.g. such dyes as mentioned in "Dyeing and Chemical Technology of Textile Fibers", Trotman, 34th Ed., C. Griffin & Co., London and "The Chemistry of Synthetic Dyes", Vankataramon (Ed.), Academic Press, New York, 1979, the disclosures of which are incorporated herein by reference.

[0039] The signal component can be capable of reacting with a protein, such as BSA and/or, for alternative embodiments described below, capable of reacting with an unreacted reactive moiety of a linker component. Furthermore, the signal component, upon reacting or binding to the spacer, should not confer any undesirable properties of the resulting water-soluble intermediate conjugate, i.e. the signal component should not promote any uncontrollable non-specific binding nor inhibit the activity of the targeting components (e.g. antibodies) bound to the conjugate.

Furthermore, the signal component should not reduce the water solubility of the conjugate significantly.

[0040] As stated earlier, only a small fraction of the reactive moieties of the linking component of the second water-soluble intermediate reacts with the signal component in the formation of the water-soluble intermediate conjugate. Depending on the signal component, the spacer component, and on the linker component, after reacting the signal component, and relative to the amount of unreacted reactive linking component available in the second water-soluble intermediate precursor, from 50 to 100% of the unreacted reactive moieties of the linker component, for example 60-100%, particularly 70-100%, such as ranging from 80-100% and notably 90-100% remain unreacted (N.B. as compared to the second water-soluble intermediate precursor).

[0041] Depending on the particular dye, the conjugate prepared by the method of the invention reflects, scatters, or emits photons in the visible range, in the UV range or in the near infrared range. Use of a visual dye such as rhodamine will cause the conjugate of the invention to reflect or scatter photons in the visible region (e.g. blue), resulting in the transmission of the complementary wavelength of color (e.g. red) to an observer. Alternatively, the use of a fluorescent dye will (when radiated) cause the conjugate of the invention to emit photons at a specific wavelength due to the return of electrons to the ground state. A "visual dye" is a dye that reflects or scatters light in the visible range.

[0042] In one embodiment the signal component is a donor/acceptor dye pair. Donor/acceptor dye pairs are known in the chemical arts. In resonant energy transfer, the donor molecule absorbs a photon and initiates energy transfer to the acceptor. The acceptor receives the energy transfer and emits a photon. The donor dye and acceptor dye can perform fluorescent resonance energy transfer (FRET) upon excitation. Some examples of suitable donor/acceptor pairs are 6-carboxyfluorescein/6-carboxy-X-rhodamine (FAM-ROX), 3-(epsilon-carboxypentyl)-3'-ethyl-5,5'-dimethyloxacarbocyanine/ 6-carboxy-X-rhodamine (CYA-ROX), and the 4,4-difluoro-4-bora-3 alpha,4 alpha-diaza-s-indacene-3-propionic acid (BODIPY) derivatives, 5,7-dimethyl-BODIPY/5-(4-phenyl-1,3-butadienyl) BODIPY (BODIPY503/512-BODIPY581/591). These donor/acceptor pairs are provided by way of example and the person of ordinary skill with reference to the present disclosure will be able to identify more donor/acceptor dye pairs that exhibit FRET

and are suitable use in the present invention. FRET is fluorescent resonance energy transfer, which is the transfer of the excited state energy from a donor to an acceptor.

[0043] In one embodiment, the spacer is covalently attached to the carrier via the linker; the signal component is a dye (e.g., a member of a donor/acceptor pair) covalently attached to the spacer; and the ligand or targeting element for a ligand is covalently attached to the carrier. The carrier is dextran; the linker is divinylsulfone; and the spacer is bovine serum albumin.

[0044] As stated earlier, and as will be understood from the examples provided herein, the method is also suitable for the preparation of water-soluble cross-linked conjugates wherein the signal component is covalently attached to the linking component, which in turn is attached to the carrier component, i.e. no protein or polypeptide spacer component is incorporated in the conjugate (vide infra). Further details are available in U.S. Patent No. 6,627,460, column 12 *inter alia*. The term "reversible precipitate" indicates that the precipitate formed is capable of being re-dissolved upon dilution with aqueous solution at 25 °C.

Targeting Elements for a Ligand to be Detected

[0045] The term "targeting element" refers to molecules, especially molecules of biological origin, which are capable of binding to or reacting with, a complementary molecule or a complementary structural region of a material of biological origin. When the targeting element is a targeting element for the ligand to be detected, the targeting element binds to or reacts with the ligand to be detected.

[0046] Examples of relevant targeting elements for a ligand to be detected are, for example: monoclonal and polyclonal antibodies, gene probes, natural and synthetic oligo- and polynucleotides, natural and synthetic mono-oligo- and polysaccharides, lectins, avidin, streptavidin, biotin, growth factors, hormones, receptor molecules, protein A and protein G; and mixtures thereof. Particular examples include anti-human Chorionic Gonadotropin (anti hCG), leutinizing hormone (LH), Rabbit anti human CRP, streptavidin, avidin, anti HIV, anti hepatitis C, anti Chlamydia, anti herpes, anti thyroid stimulating hormone (anti TSH), anti-Listeria, and anti-salmonella.

Ligands to be Detected

[0047] "Ligands" are molecules to which targeting elements for the ligand will bind. Examples of ligands useful in the present invention are antigens and haptens, but can include any ligand of interest in the detection. Examples of

hormones as ligands, are steroid hormones (e.g. estrogen, progesterone or cortisone), amino acid hormones (e.g. thyroxine) and peptide and protein hormones (e.g. vasopressin, bombesin, gastrin or insulin), and drugs of abuse. A “drug of abuse” is a drug that is taken for non-medicinal reasons (usually for mind-altering effects). The abuse of such drugs can lead to physical and mental damage and (with some substances) dependence and addiction. Examples of drugs of abuse include cocaine, amphetamines (e.g., black beauties, white bennies, dextroamphetamines (dexies, beans), methamphetamines (crank, meth, crystal, speed)), barbiturates, lysergic acid diethylamide (LSD), depressants, sedatives (e.g., selective serotonin reuptake inhibitors), phencyclidine (PCP), tetrahydrocannabinol (THC), and opiates (e.g., morphine, opium, codeine, and heroin).

[0048] The present invention also provides water-soluble conjugate prepared according to any method of the invention.

[0049] In another aspect the present invention provides methods of preparing a water-soluble conjugate involving preparing a water-soluble conjugate as described herein as a precipitate in a suspension. A pellet containing the water-soluble conjugate is separated from the suspension, and the pellet washed with an aqueous solution to form a second suspension. A pellet is separated from the second suspension containing the water-soluble conjugate. The water-soluble conjugates prepared according to these methods can have the same structure as those conjugates described above. For example, the conjugates can further contain a spacer, and the carrier can be covalently attached to the linker, and the signal component covalently attached to the spacer.

[0050] In one embodiment the water-soluble conjugate is purified by the process of separating the precipitate from a supernatant, forming a suspension of the precipitate in an aqueous solution, and separating the precipitate from a supernatant. The conjugates can contain a non-specific protein attached to the carrier via the linker (e.g., bovine serum albumin, an immunoglobulin). In one embodiment the targeting element is an antibody that has been treated with a reducing agent. By “reducing agent” is meant a substance that chemically reduces other substances by donating an electron or electrons. Examples of reducing agents include beta-mercaptoethanol, dithiothreitol, and 2-iminothiolane.

[0051] In another aspect, the present invention provides water-soluble conjugates containing a carrier, a linker covalently bound to the carrier, a signal

component, a targeting element for a ligand to be detected or a ligand to be detected, and a non-specific protein. In one embodiment the non-specific protein is covalently bound to the carrier via the linker. In another embodiment the non-specific protein is bound to the carrier via the linker and to no other component (other than the linker) of the conjugate. In other embodiments at least 2% or at least 3% or at least 5% or at least 10% or at least 15% or at least 20% of the non-specific proteins are bound to the carrier via the linkers and to no other component (other than the linker) of the conjugate. Any of the water-soluble conjugates can also contain a spacer component.

[0052] In various embodiments the "non-specific protein" is a protein that does not have a binding specificity or target within the context it is used. The non-specific protein is typically linked to the water-soluble conjugate by a linking chemistry. Bovine serum albumin, immunoglobulins, keyhole limpet hemocyanin, and other proteins are examples of non-specific proteins. In one embodiment the non-specific protein is a protein other than the one used as a spacer (when a spacer is present), but the spacer and non-specific protein can also be the same protein used to accomplish different functions. The non-specific protein can contain amino groups, which are used to covalently bind the non-specific protein to the conjugate, although other suitable linking chemistries may also be used. In another embodiment, the spacer and the non-specific protein are independently and covalently attached to the carrier via the linker; the signal component is covalently attached to the spacer; and the ligand to be detected or targeting element for a ligand to be detected is covalently attached to the carrier.

[0053] In other embodiments the signal component is attached to either or both of the spacer and/or the non-specific protein. It is also possible to attach the non-specific protein and spacer to the carrier via the linker, and attach the targeting element or ligand, and signal component to either or both of the non-specific protein and spacer.

[0054] In another aspect, the present invention provides methods of preparing a water-soluble conjugate involving contacting a water-soluble intermediate precursor having at least one carrier and at least one linker, with at least one targeting element for a ligand to be detected or a ligand to be detected, and a non-specific protein. A signal component is also attached to form the final conjugate. A suspension is formed containing a precipitate of the water-soluble conjugate, and the water-soluble conjugate is extracted from the suspension. In various embodiments the non-specific

protein can be bovine serum albumin, an immunoglobulin, or keyhole limpet hemocyanin. The carrier and linkers can also contain the signal component before addition of the targeting element or ligand, and non-specific protein, or the signal component can be added after attaching the targeting element or ligand and non-specific protein. In one embodiment the targeting element or ligand, and non-specific protein are contacted, added, or attached simultaneously to the water-soluble intermediate precursor. The targeting element or ligand and non-specific protein can be attached to the intermediate precursor at a salt concentration of at least 1.6 M or at least 1.7 M or at least 1.8 M or at least 1.9 M or at least 2.0 M or at least 2.2 M or at about 2.5 M. The non-specific protein can be reacted with the precursor at ratios of 1:1 or greater (precursor to non-specific protein) or 1:5 or greater, or 1:7 or greater, or 1:10 or greater, or 1:12 or greater, or 1:15 or greater, or 1:20 or greater. The method produces a water-soluble conjugate as described herein.

[0055] In another embodiment the conjugate can be created by attaching the non-specific protein to the carrier via the linker so that all linkers are blocked. The targeting element or ligand can then be attached to the precursor via the non-specific protein. The signal component can be attached either with the targeting element or ligand and non-specific protein, or can be attached in a later step to form the final conjugate.

[0056] In another aspect, the present invention provides methods for preparing a water-soluble conjugate involving a) contacting a water-soluble intermediate conjugate having a carrier, a linker, a spacer, and a signal component, with i) a targeting element for a ligand to be detected or ii) a ligand to be detected, to form a suspension containing a precipitate comprising the water-soluble conjugate. The water-soluble conjugate is extracted from the suspension. In this aspect the targeting element for a ligand to be detected or the ligand to be detected is pre-treated with a reducing agent prior to contact with the water-soluble intermediate conjugate. By "pre-treat" is meant that the composition is contacted or incubated with the reducing agent. In one embodiment the reducing agent is dithiothreitol, which can be used at any suitable concentration. For example, the pre-treatment can be with at least about 15 mg of dithiothreitol/100 ul, or at least about 10 mg/100 ul, or at least about 5 mg/100 ul, or at least about 20 mg/100 ul. Equivalent quantities of other reducing agents can also be used.

Devices

[0057] In another aspect the invention provides a device containing a water-soluble conjugate of the invention. The conjugate is located on a test strip, which is a porous carrier material having a sample zone and a detection zone. Liquid sample applied to the sample zone flows to the detection zone. The test strip also has a second targeting element selective for a targeting element bound to the ligand or for a ligand suspected to be present in the liquid sample, applied to the detection zone of the test strip. By "porous carrier" is meant a bibulous material through which fluid can move by capillary force. An example of such a material is nitrocellulose, although persons of ordinary skill in the art will identify other bibulous materials that also function in the invention, for example, polyamide, and pretreated papers. The "sample zone" is the area of the test strip where sample to be tested is applied. A "reagent zone" is an area where reagents are contained on the test strip. The reagents can be present in a dried form, and can be movably present on the strip. A "detection zone" is the area of the test strip where a measurement is taken to determine the presence, absence, or amount of a ligand suspected to be present in the sample. The device can also have a "label zone" where label is movably applied to the test strip. "Capillary force" refers to the interfacial forces that act among liquids in a capillary or in a porous medium, and which cause liquid to move through the capillary or porous medium. By "movably" is meant that the reagents or other composition can be moved along the device from one zone to another by the flow of liquid through the test strip.

[0058] Various embodiments of the devices can be provided. In one embodiment the water-soluble conjugate is in a dry form on the test strip, upstream of the detection zone and downstream of the sample zone. The test strip can also have a control zone, which can be located downstream from the detection zone. The "control zone' contains a targeting element, and binding at the control zone indicates that the assay is functioning as designed. In another embodiment the device also has a casing, which envelopes the test strip and defines the sample zone. The porous carrier can be backed with a moisture-impervious material, which is placed in contact with the inside of the casing. In one embodiment the device also has a cap, which is selectively received over one end of the casing and covers the sample zone of the device. The casing can be made of plastic or another suitable material. In one embodiment the test strip also contains a filter, which is situated upstream from the detection zone, and can be a part of the porous carrier material. The filter serves to

remove any contaminating matter that may be present in the applied sample. The test strip can also be prepared so it has a portion of the binding sites within the test strip blocked with a blocking protein or polyvinyl alcohol. For example, the blocking protein can be bovine serum albumin, milk protein, or another material having an equivalent effect on the assay. The sample applied to the device can be urine, serum, plasma, blood, semen, sputum, or another body fluid or other fluid of biological origin to be tested. In another embodiment the device has a test strip, a casing, and a portion of the test strip protrudes from the casing. For example, a 1 cm or less portion of the test strip protrudes from the casing for receiving sample.

[0059] In another aspect the present invention provides methods for determining the presence an analyte in a liquid sample. The method involve contacting a liquid sample to a portion of the test device of the invention, the portion being located upstream from the detection zone; allowing the liquid sample to flow to the detection zone; and determining the presence, absence, or amount of the analyte in the liquid sample by observing the detection zone. The detecting step can be visually observing the signal in the detection zone.

[0060] In another aspect the present invention provides methods for preparing a water-soluble conjugate involving a) contacting a water-soluble intermediate precursor containing a carrier, a linker, a spacer, and a targeting element for a ligand to be detected or a ligand to be detected, with a signal component to form a suspension containing a precipitate comprising the water-soluble conjugate. Water-soluble conjugate is then extracted from the suspension.

[0061] The summary of the invention described above is not limiting and other features and advantages of the invention will be apparent from the following detailed description, as well as from the claims.

Detailed Description of the Invention

[0062] The present invention provides methods of preparing water-soluble conjugates that offer surprising and beneficial effects. It was discovered unexpectedly by the present inventors that by appropriate sonication of the water-soluble conjugate after cross-linking, higher product yields are obtainable. The sonication process produces a clear solution, meaning that it contains no non-liquid matter visible with

the unaided eye, or produces minimal non-liquid matter. Normally, after following this procedure, further centrifugation is unnecessary.

[0063] Another aspect of the invention involves the washing of a pellet produced by centrifugation of a formed water-soluble conjugate, which is present as a precipitate in the reaction product. The supernatant is separated from the pellet and the pellet washed with an aqueous solution or buffer to form a second suspension. A second pellet is separated, and the wash step can be repeated 1-2 more times, as necessary. It was discovered that the steps of washing the pellet solubilized free (unreacted) targeting element (e.g., an antibody), which is found in the supernatant. Thus, unreacted targeting element can then be easily disposed of. It was also found that the steps of washing eliminated the need to perform further purification of the product. Thus, the expensive step of purification of the product on, for example, a gel filtration (S-300) column was eliminated.

[0064] In another aspect the present invention provides water-soluble conjugates (and methods of their preparation) which contain a carrier, a linker, optionally a spacer component, a signal component, a targeting element for a ligand to be detected or a ligand to be detected, and a non-specific protein covalently bound to the carrier via the linker. Previously, large quantities of targeting element (or ligand) molecules were utilized to ensure sufficient coupling and cross-linking to produce the water-soluble conjugate. The present inventors discovered that substantially less targeting element or ligand is necessary to ensure sufficient cross-linking than is used by binding to all available sites on the carrier. By including a non-specific protein in the reaction mixture, many sites on the carrier (available through the linker) will become occupied by the non-specific protein. Yet sufficient binding of targeting element or ligand will occur to result in a useful product. Thus, by utilizing the methods taught herein, the user is able to reduce the amount of targeting element (or ligand) utilized in the preparation procedure, and therefore substantially reduce the cost of producing the product.

[0065] In another aspect the present invention provides methods of preparing a water-soluble conjugate by contacting a water-soluble intermediate conjugate with a targeting element or ligand to form a suspension containing a precipitate that contains the water-soluble conjugate, extracting the water-soluble conjugate from the suspension, where the targeting element or ligand is pre-treated with a reducing agent prior to contact with the water-soluble intermediate conjugate. It was discovered

unexpectedly that by pre-treating the targeting element with a reducing agent, a higher binding rate of the targeting element to the carrier is achieved, resulting in an increase in the sensitivity of the assay. Example 5 provides a practical application of this aspect of the invention. Any reducing agent can be used, for example, dithiothreitol, beta-mercaptoethanol, Traut's Reagent (2-iminothiolane), or another reducing agent.

[0066] The present methods and compositions are useful in a variety of assay formats. For example, some formats utilize an antibody that is specific for the ligand suspected of being present in the sample. In these formats, reagents can be movably located on the test strip and sample is applied to the sample zone. Sample then migrates through the reagent zone where reagents can bind to ligand suspected of being present in the sample, and arrives at the detection zone where a targeting element is applied to the zone and binds to the ligand suspected to be present or the targeting element bound to the ligand, or even to another component of the conjugate. By "selective binding" is meant that the targeting element distinguishes between the ligand of interest from any other ligand likely to be present in the sample, so that the assay functions as intended. A targeting element that binds selectively can still bind to more than one ligand. By "specific binding" is meant that the targeting element binds to its target ligand and to no other ligand that may be present in the sample.

[0067] In another assay format two antibodies can be used which have a lower selectivity for the ligand of interest, and therefore can bind not only to the ligand of interest, but also to a second molecule present in the sample. In this format a scavenger antibody is used that binds to the binding sites on the second molecule, thus blocking these sites and leaving the two antibodies to bind only to the ligand of interest. In other formats, more than one scavenger antibody can be used and more than two antibodies can be used. With reference to the present disclosure the person of ordinary skill will be able to devise additional assay formats, which are also contemplated in the present invention. The particular formats listed herein are provided by way of example.

[0068] The invention can also be applied in a direct sandwich assay format. In this format, sample is applied to a sample zone, and flows through a label zone containing a label (e.g., dextran-BSA-anti-hCG Antibody-rhodamine) and if the ligand to be detected is present the label binds to the ligand. Sample then continues to flow to the detection zone (containing, for example, anti-hCG antibody affixed to the

test strip). In the detection zone labeled ligand is bound to the detection zone and observation of the detection zone provides the result of the assay.

[0069] In another assay format (sometimes called an "indirect" format), sample is applied to a sample zone and migrates through a reagent zone containing a targeting element specific for the ligand to be detected movably present in the reagent zone (e.g. biotin-hCG Ab). The sample then continues to migrate through a label zone, which contains a conjugate of the invention that specifically binds to the ligand (e.g., the beta-portion of hCG) or to a targeting element bound to the ligand. Thus, a signal component is attached to the ligand to be detected. The sample then continues to migrate to the detection zone, where there is a bound a targeting element for the sample (e.g., streptavidin-IgG or streptavidin-BSA). Visual observation of the detection zone reveals the presence, absence, or amount of ligand present.

[0070] With reference to the present disclosure the person of ordinary skill in the art will realize other formats for application of the invention, which are also contemplated here. For example, the present invention can be applied in the formats described in the following references: 5,602,040, 5,622,871, 5,656,503, 6,187,598, 6,228,660, 6,352,862, 2001/0,008,774, 2001/0,041,368, 5,714,389, 5,989,921, 6,485,982, 5,252,496, 5,559,041, 5,728,587, 6,027,943, 6,506,612, 6,541,277, 2002/0,160,525, 5,073,484, 5,654,162, 6,020,147, 5,120,643, 5,578,577, 6,534,320, 4,703,017, 4,743,560, 5,591,645, 3,011,874, 3,641,235, 4,094,647, 4,168,146, 4,373,932, 4,477,575, 4,722,889, 4,861,711, 4,943,522, 4,978,503, 5,571,726, EP 0,149,168, EP 0,170,375, EP 0,192,320, EP 0,250,137, EP 0,287,731, EP 0,291,194, EP 0,349,215, GB 2,062,224, GB 8,709,873. The following examples are provided for further illustration of the invention.

Example 1 – Preparation of Water-Soluble Conjugate

[0071] This embodiment of the preparation procedures involves four steps: activation of the dextran with divinyl sulfone, attaching BSA to the activated dextran, incorporating the rhodamine dye to the BSA portion of the dextran-BSA backbone, and cross-linking the antibody to the dextran-BSA-Rhodamine backbone.

<u>Activated dextran:</u>

[0072] The following solutions were prepared for the activation: 25 mg/ml dextran (500,000 MW) in distilled water, 0.5 M potassium phosphate pH 11.4, and 25 mg/ml sodium borohydride in distilled water (prepared just prior to using).

[0073] The activation conditions were 10 mg/ml final concentration of dextran, 0.25 M potassium phosphate buffer, 0.25 mg/ml final concentration of sodium borohydride and 5% DVS. The entire operation was done in a fume hood. The dextran, distilled water and potassium phosphate buffer were combined initially and allowed to mix for 10-15 minutes. The sodium borohydride was added and followed immediately by the DVS. The timer was started from the first drop of DVS added and the DVS was added in a dropwise fashion within 2 minutes. After the entire amount of DVS was added, the solution was continually stirred for up to 30-35 minutes. After the 30-35 minute incubation, the activation was stopped by adjusting the pH to 7 with 25% HCl. The activated dextran was dialysed extensively against distilled water, with the water being changed twice a day for four days. The dialysate was collected, and chlorobutanol added at a final concentration of 0.01%.

<u>Attachment of BSA to activated dextran:</u>

[0074] The solutions prepared for the conjugation were: 50 mg/ml BSA (bovine serum albumin) in 0.1 M sodium chloride, 0.4 M potassium phosphate pH 10.4 and 0.1 M sodium chloride.

[0075] The conjugation conditions were: 1:25 activated dextran to BSA molar ratio, 0.010M K_2HPO_4, pH 10.4, 30°C, and 22 hours. The activated dextran, BSA solution and potassium phosphate buffer were added together. The pH of the mixture was adjusted to 10.4 with 1M HCl. The mixture was placed in a 30°C oven for 22 hours. After the 22 hours incubation, the pH of the mixture was lowered to 6.5 with 1 M HCl. Then the mixture was purified using a S300 size exclusion column with 0.1 M sodium chloride as the running buffer. The first peak was collected and used for the next step.

<u>Incorporation of the Rhodamine dye to the BSA portion of the Dextran-BSA:</u>

[0076] The following solutions were prepared: 1 M sodium bicarbonate pH 8.6, 10 mg/ml rhodamine isothiocyanate in DMSO, 0.5M K2HPO4 pH 7.2.

[0077] The conjugation conditions were 100-200 ug dye/mg BSA, 0.1 M sodium bicarbonate, pH 8.0, 30°C, and 1 hour. The dextran-BSA, rhodamine solution, and sodium bicarbonate buffer were added together. The pH was adjusted to 8.0 with 1 M HCl. The mixture was incubated in a 30°C oven for 1 hour. After the incubation, the mixture was dialysed extensively against 10 mM K_2HPO_4 pH 7.2 (2 changes per day for 4 days). The dialysate was collected and Bronidox added at a final concentration of 0.05%.

Cross-linking of Antibody to Dextran-BSA-Rhodamine:

[0078] The components needed for the cross-linking are: antibody solution, 3.5 M K_2HPO_4 pH 9-10, dextran-BSA-Rhodamine, 0.1 M cysteine in distilled water (prepared just prior to use), distilled water and 50 mM Tris pH 7.2/0.1M NaCl/0.02% sodium azide.

[0079] The cross-linking conditions were 1:2.5 to 1:5 dextran-BSA-rhodamine to antibody molar ratio, 30 °C, 18-22 hours and 2.5 M K_2HPO_4 salt molarity. The dextran-BSA-rhodamine was centrifuged at 4000 g to remove any particulates. The antibody solution, dextran-BSA-rhodamine and K_2HPO_4 were combined together. The mixture was incubated in a 30°C oven for 18-22 hours. After the incubation, cysteine was added at 1/10 the total volume. The salt concentration was adjusted from 2.5 M to 1.75 M by adding distilled water. Then the mixture was centrifuged at 9,333 g to pellet the water soluble conjugate. The pellet was resuspended in distilled water at ½ the original volume of dextran-BSA-rhodamine used for the cross-linking. The resuspended pellet was centrifuged at 327g for 5 minutes. The supernatant was purified in a S300 gel filtration column using 50 mM Tris/0.1M NaCl/0.02% sodium azide as the running buffer. The first peak was collected and used as a label conjugate.

Example 2 – Use of Sonication After Cross-Linking Antibody to Dextran-BSA-Rhodamine

[0080] The example illustrates the use of sonication in the methods. The cross-linking conditions were 1:2.5 dextran-BSA-Rhodamine to antibody molar ratio, 30°C, 18-22 hours and 2.5M salt molarity. The dextran-BSA-Rhodamine was centrifuged at 4000 g to remove any particulates. The antibody solution, dextran-BSA-Rhodamine and K_2HPO_4 were combined together. The mixture was incubated

in a 30°C oven for 18-22 hours. After the incubation, cysteine was added at 1/10 the total volume. The salt concentration was adjusted from 2.5 M to 1.75 M by adding distilled water. The mixture was then centrifuged at 9,333 g to pellet the water soluble conjugate. The pellet was resuspended in distilled water at ½ the original volume of dextran-BSA-Rhodamine used for the cross-linking. The resuspended pellet was sonicated (power set at 700 watts, 5 second /cycle, 10 cycles, 10 second pause between cycles) and then centrifuged at 327 g for 5 minutes. The supernatant was purified in a S300 gel filtration column using 50 mM Tris/0.1 M NaCl/0.02% sodium azide as the running buffer. The first peak was collected and used as a label conjugate.

[0081] For the preparation of a label pad, an OD 1.5 was used at 27-ul/test. Results showed a negative result when no ligand was present, and a positive result when 25 mIU/ml and 50 mIU/ml of ligand was present.

Example 3 – Use of Washing Procedure Eliminates Gel Filtration Column

[0082] The following procedure illustrates that washing of the pellet after precipitation eliminates the necessity of purification of the water-soluble conjugate by gel fitration or another step.

[0083] The following solutions containing antibody and "Dex-BSA-Rhodamine" were prepared: 0.00258 umole antibody and 0.00535 umole dextran (as "dex-BSA-rhodamine") was mixed with 3.5 M potassium phosphate buffer, pH 11.5, to arrive at the following final concentrations: 2.5 M potassium phosphate buffer, pH 11.0. The molar ratio in the solution: "dex-BSA-rhodamine"/antibody was 1 / 2.5.

[0084] After mixing, a precipitate was observed in solution. Coupling was continued at 30 °C for 3 hours. After coupling, cysteine was added to the samples to a final concentration of 0.01 M cysteine. The concentration of phosphate buffer in solution was adjusted to 1.75 M by addition of de-ionized water to the solution. Solution was then spun for 5 minutes at 10,000 rpm and the supernatant, which was clear and almost colorless, was carefully aspirated with a pipette.

[0085] The precipitate (pellets) containing free antibody and coupled antibody was dissolved in 3 ml de-ionized water. The re-dissolved precipitate was spun for 10 minutes at 12000 rpm; the supernatant containing free antibody was discharged. The above step was repeated once. The precipitate (pellets) was then dissolved in 1 ml

deionized (DI) water. The OD_{558} of the "Dex-BSA-Rhodamine-Antibody" conjugates was measured and was greater than 20. Results are summarized below:

Results: OD $_{558/280}$ = 41/39. Used OD=20 to make label pad. Volume=120ul

	1 IU hCG/ml	50 mIUhCG/ml	25 mIUhCG/ml	Urine(-)	DI water
Sample-1	+++	++	+	-	-
Sample-2	+++	++	+	-	-

Example 4 – Preparation of Water-Soluble Conjugate With Non-Specific Protein

[0086] This example illustrates the preparation of water-soluble conjugate using a non-specific protein, in this case BSA and immunoglobulin.

Method:

(1) The following materials were added in this order:
Monoclonal anti-beta HCG from Medix, clone 5008 10 mg
Dextran –BSA-Dye 6 ml (Dextran conc. 0.0043 um/ml), (Dex: Ab = 1:2.5)
Mouse IgG from Acon Bio, R103008, 5 mg (Dex: mouse IgG = 1: 1.25) or without Mouse IgG
3.5 M K2HPO4 at pH 9.5 20.2 ml (final 2.5 M)
30C, O/N

0.1 M cysteine 2 ml
DI water 6.67 ml
8000 RPM, 10 minutes
S-300 purification
Applied purified Ab conjugate to Label pad with OD 558 = 0.686, 59 ul per tests

Test result:

	With Mouse IgG	Without mouse IgG
Negative urine	-	-
HCG 25 mIU/ml	+	+
HCG 50 mIU/ml	+	+

2) In a second example, the following materials were added in this order:

	1	2	3	4	5	6	7	8
Ab	5mg	5mg	2mg	1mg				
BSA	0	4.224mg	6.8mg	7.66mg				
Dex-BSA-rhodamine	0.0128 um	0.0128 um	0.0128 um	0.0128 um				
Dex:Ab	1:2.5	1:2.5	1:1	1:0.5				
Dex:BSA	1:0	1:5	1:8	1:9				
K2HPO4	2.5M, pH 10							
Temperature	30C							
Time	16 hours							
10000RPM, 10minutes	Partial Soluble	soluble	soluble	soluble				
Purification	S-300							
	Not finished	done	done	Lost				

Applied purified Ab conjugate to Label pad with OD 558 = 0.686, 59 ul per tests

	With BSA (1:5)	With BSA (1:8)
Negative urine	-	-
HCG 25 mIU/ml	+	+
HCG 50 mIU/ml	+	+

Example 5 – Preparation of Water-Soluble Conjugate by Pre-Treatment with Dithiothreitol

[0087] This example illustrates the preparation of a water-soluble conjugate using pre-treatment of antibody (targeting element) with dithiothreitol.

[0088] Dex-BSA-Rhodamine, dex conc. 0.00464 uM/ml, Ab: Monoclonal anti-beta hCG , clone 5008, 4.8mg/ml.

2. Method

	1	2	3a	3b	4
Ab	4mg	4mg	4mg	4mg	2mg
DTT 15.4mg/100ul	17ul	34ul	80ul	80ul	0
RT	30'	1h15'	1h30'	1h30'	no
Purification with Column PD10	Yes	Yes	Yes	Yes	no
Dex-BSA-Dye	1.1123 ml	1.1123ml	1.1123ml	1.1123ml	1.1123ml
Dex:Ab	1:5	1:5	1:5	1:5	1:2.5
3.5MK_2HPO_4	7.03ml (2.5M)	6.53ml (≈2.3M)	6.52ml (≈2.3M)	6.52ml (2.5M)	3.8225ml (2.5M)
Buffer pH	9	9	9	8.5	9
30C 11.5h	Yes	Yes	Yes	Yes	Yes
Cystein 1/10 volume	Yes	Yes	Yes	Yes	Yes
DI water to final salt Conc.	1.75 M	1.75 M	1.75 M	1.75 M	1.75 M
10000RPM, 10 minutes	Precipitate	Precipitate	Precipitate	Precipitate	Precipitate
D.I water	0.6 ml	0.6 ml	0.6 ml	0.6 ml	0.6ml
3000 RPM x 5 minutes	Most precipitate	soluble	soluble	soluble	soluble
S-300 purification	Yes	Yes	Yes	Yes	Yes
Label pad OD1.5 /27ul/test					

Result
NC: FHC 102 stripped with Monoclonal anti alpha hCG, Acon Bio

Sample	LP 2	LP 3a	LP 3b	LP 4
DI water	-	-	-	-
Negative urine	-	-	-	-
hCG 25 mIU/ml	+ (L3)	+(L3)	+ (L4)	+(L5)
hCG 50 mIU/ml	+(L4)	+ (L4)	+(L4)	+(6)
hCG 100 mIU/ml	+	+	+	+
hCG 1 IU/ml	+	+	+	+

Example 6 – Alternate Stepwise Conjugates

[0089] This example illustrates one alternate method of preparing the water-soluble conjugate. In this method, the signal component is linked to the targeting element prior to the combination with the water-soluble intermediate conjugate to form the water-soluble conjugate.

[0090] The bovine serum albumin was conjugated to activated dextran. The composition was purified to separate the free BSA. The hCG antibody was then conjugated to dextran-BSA at a molar ratio of 5:1 in 0.1 M potassium phosphate, pH 9.6, at 30 °C for 18 hours. The composition was again purified to separate the free antibody. Rhodamine dye was conjugated to the dextran-BSA-antibody at a ratio of 150 ug dye/mg protein in 0.1 M sodium bicarbonate, pH 8.0 at 30 °C for 3 hours. The reaction was stopped with cysteine and dialysed extensively again 10 mM K_2HPO_4 pH 7.2. Lastly, the antibody was cross-linked to the dextran-BSA-antibody-dye at a ratio of 2.5:1 in 2.5 M K_2HPO_4, pH 10.6 at 30°C for 18 hours. The conjugate was then purified to separate the free antibody.

[0091] An OD of 0.8 at 27 ul/test was used to make the label pad. Results showed a negative result when no ligand was present, and a positive result when 100 mIU/ml of ligand was present.

Example 7 – Indirect Assay Format

[0092] This example illustrates the use of the invention in an indirect assay format.

[0093] The water soluble conjugate was prepared according to the procedures described above, except that after the first centrifugation, the pellet was washed three times in distilled water. The final pellet was then resuspended in distilled water. The solution was sonicated using 5-second cycles for 10 cycles with 10-sec pause between cycles. A label pad was made at OD_{550} 45.

[0094] The label pad was evaluated according to this configuration: a test strip containing a sample zone, a biotinylated alpha-hCG antibody in the reagent zone, a label pad, and Streptavidin-IgG striped down on the nitrocellulose and absorbent in the detection zone. The test strip was placed inside a plastic housing. The test device was tested with different levels of hCG concentration, negative urine and distilled water.

[0095] The results obtained at 3 minutes were negative for distilled water and urine with no hCG. And a positive result was obtained for samples containing 1 IU/ml, 500 mIU/ml, 100 mIU/ml, and 50 mIU/ml.

[0096] The invention illustratively described herein may be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by various embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0097] The contents of the articles, patents, and patent applications, and all other documents and electronically available information mentioned or cited herein, are hereby incorporated by reference in their entirety to the same extent as if each individual publication was specifically and individually indicated to be incorporated by reference. Applicants reserve the right to physically incorporate into this application any and all materials and information from any such articles, patents, patent applications, or other documents.

Claims

We claim:

1. A method for preparing a water-soluble conjugate, comprising:

   a) preparing a water-soluble conjugate comprising
      a carrier,
      a linker, and
      a signal component, and
      a targeting element for a ligand to be detected or a ligand to be detected,
   as a reversible precipitate in a suspension;

   b) subjecting the suspension to sonication to form a sonicated formulation; and

   c) separating a supernatant containing the water-soluble conjugate from the sonicated formulation.

2. The method of claim 1 wherein the water-soluble conjugate further comprises a spacer.

3. The method of claim 2 wherein the carrier is covalently attached to the linker and the signal component is covalently attached to the spacer.

4. The method of claim 1, wherein the water soluble conjugate is prepared by contacting a water-soluble intermediate conjugate with the ligand to be detected or the targeting element for a ligand in the presence of a lyotropic salt at a concentration of at least about 1.25 M.

5. The method of claim 4, wherein the lyotropic salt comprises a component selected from the group consisting of: a sulphate, a phosphate, a citrate and tartrate of lithium, sodium, potassium, calcium and ammonium.

6. The method of claim 5 wherein lyotropic salt is present at a concentration of about 2.5 M and the salt is selected from the group consisting of: potassium phosphate and sodium phosphate.

7. The method of claim 1 wherein the sonication is performed at about 700 watts.

8. The method of claim 7 wherein the sonication is performed at a frequency of from 20 to 24 kHz.

9. The method of claim 1, wherein the water-soluble conjugate is separated from the supernatant by centrifugation.

10. The method of claim 1, further comprising that the water-soluble conjugate is purified from the supernatant by application to a gel filtration column.

11. The method of claim 2, wherein the carrier is selected from the group consisting of: dextran, starch, glycogen, agarose, cellulose, natural gum, and any mixture thereof.

12. The method of claim 11, wherein the carrier is dextran.

13. The method of claim 1, wherein the linker is divinyl sulfone.

14. The method of claim 2, wherein the spacer is selected from the group consisting of: a polypeptide, bovine serum albumin, ovalbumin, and a globulin.

15. The method of claim 14, wherein the spacer is bovine serum albumin.

16. The method of claim 1, wherein the signal component is a non-particulate label.

17. The method of claim 16, wherein the non-particulate label is a soluble dye.

18. The method of claim 17, wherein the dye is rhodamine.

19. The method of claim 17, wherein the non-particulate label comprises a donor dye and an acceptor dye.

20. The method of claim 19, wherein the donor dye and the acceptor dye perform fluorescent resonance energy transfer (FRET) upon excitation.

21. The method of claim 2, wherein

the spacer is covalently attached to the carrier via the linker;

the signal component is a dye covalently attached to the spacer; and

the ligand or targeting element for a ligand is covalently attached to the carrier.

22. The method of claim 21 further comprising that

the carrier is dextran;

the linker is divinylsulfone; and

the spacer is bovine serum albumin.

23. The method of claim 22 wherein the water-soluble conjugate further comprises a non-specific protein attached to the carrier via the linker.

24. The method of claim 23 wherein the non-specific protein is an immunoglobulin or bovine serum albumin.

25. The method of claim 21, wherein the targeting element for a ligand is selected from the group consisting of: a monoclonal or polyclonal antibody, a receptor molecule, a lectin, avidin, streptavidin, and biotin.

26. The method of claim 21 wherein the ligand is selected from the group consisting of: an antigen, a hapten, a gene probe, an oligo- or polynucleotide, a mono-, oligo- or polysaccharide, a hormone, a pharmacological agent, a peptide, a lectin, avidin, streptavidin, biotin, a growth factor, a protein, or any mixture thereof.

27. The method of claim 21, wherein the ligand is selected from the group consisting of: a bacterial or viral antigen, human chorionic gonadotropin (hCG), luteinizing hormone (LH), and a drug of abuse.

28. The method of claim 25, wherein the targeting element is an antibody that has been treated with a reducing agent.

29. A method of preparing a water-soluble conjugate, comprising:

preparing a water-soluble conjugate comprising:

a carrier,

a linker,

a signal component; and

a targeting element for a ligand to be detected, or a ligand to be detected,

as a precipitate in a suspension;

separating a pellet containing the water-soluble conjugate from the suspension;

washing the pellet with an aqueous solution to form a second suspension;

separating a second pellet containing the water-soluble conjugate from the second suspension.

30. The method of claim 29 wherein the water-soluble conjugate further comprises a spacer.

31. The method of claim 30 wherein the carrier is covalently attached to the linker and the signal component is covalently attached to the spacer.

32. The method of claim 30, further comprising that the water soluble conjugate is prepared by contacting a water-soluble intermediate conjugate comprising a carrier, a linker, a spacer, and a signal component with i) a targeting element for a ligand to be detected, or ii) a ligand to be detected to form the reversible precipitate, in the presence of a lyotropic salt at a concentration of at least about 1.25 M.

33. The method of claim 32, wherein the lyotropic salt comprises components selected from the group consisting of: a sulphate, a phosphate, a citrate or tartrate of lithium, sodium, potassium, calcium and ammonium, and mixtures thereof.

34. The method of claim 33 wherein lyotropic salt is present at a concentration of about 2.5 M and the salt is selected from the group consisting of: potassium phosphate and sodium phosphate.

35. The method of claim 30, wherein second pellet containing the water-soluble conjugate is separated from the second suspension by centrifugation.

36. The method of claim 30, further comprising that the water-soluble conjugate is purified by the process of separating the precipitate from a supernatant, forming a suspension of the precipitate in an aqueous solution, and separating the precipitate from a supernatant.

37. The method of claim 30, wherein the carrier is selected from the group consisting of: dextran, starch, glycogen, agarose, cellulose, natural gum, and mixtures thereof.

38. The method of claim 37, wherein the carrier is dextran.

39. The method of claim 30, wherein the linker is divinyl sulfone.

40. The method of claim 30, wherein the spacer is selected from the group consisting of: a protein, a polypeptide, bovine serum albumin, ovalbumin, or a globulin.

41. The method of claim 40, wherein the spacer is bovine serum albumin.

42. The method of claim 29, wherein the signal component is a dye other than a particulate dye.

43. The method of claim 42, wherein the label is a visual dye.

44. The method of claim 42, wherein the dye is rhodamine.

45. The method of claim 29, wherein the signal component comprises a donor dye and an acceptor dye.

46. The method of claim 45, wherein the donor dye and the acceptor dye perform fluorescent resonance energy transfer (FRET) upon excitation.

47. The method of claim 30, wherein

the carrier is covalently attached to the spacer via the linker;

the signal component is covalently attached to the spacer; and

the ligand or targeting element for a ligand is covalently attached to the carrier.

48. The method of claim 47 wherein the water-soluble conjugate further comprises a non-specific protein attached to the carrier via the linker.

49. The method of claim 48 wherein the non-specific protein is covalently attached to the carrier via the linker.

50. The method of claim 48 wherein the non-specific protein is an immunoglobulin.

51. The method of claim 47, wherein the targeting element for a ligand is selected from the group consisting of: a monoclonal or polyclonal antibody, a receptor molecule, a lectin, avidin, streptavidin, and biotin.

52. The method of claim 47 wherein the ligand is selected from the group consisting of: an antigen, a hapten, a gene probe, an oligo- or polynucleotide, a mono-, oligo- or polysaccharide, a hormone, a pharmacological agent, a peptide, a lectin, avidin, streptavidin, biotin, growth factors, a protein, and any mixture thereof.

53. The method of claim 29, wherein the ligand is selected from the group consisting of: a bacterial or viral antigen, human chorionic gonadotropin (hCG), luteinizing hormone (LH), and a drug of abuse.

54. The method of claim 30, wherein the targeting element is an antibody that has been treated with a reducing agent.

55. A water-soluble conjugate prepared according to the method of claim 30.

56. A water-soluble conjugate prepared according to the method of claim 54.

57. A water-soluble conjugate comprising:

a carrier,

a linker covalently bound to the carrier,

a signal component;

a ligand to be detected or a targeting element for a ligand to be detected; and

a non-specific protein covalently bound to the carrier via the linker, and to no other component of the water-soluble conjugate.

58. The water-soluble conjugate of claim 57 further comprising a spacer component.

59. The water-soluble conjugate of claim 58 wherein the non-specific protein is an immunoglobulin or bovine serum albumin.

60. The water-soluble conjugate of claim 59 wherein the carrier is selected from the group consisting of: dextran, starch, glycogen, agarose, cellulose, natural gum, and mixtures thereof.

61. The water-soluble conjugate of claim 58, wherein the carrier is dextran.

62. The water-soluble conjugate of claim 61 wherein the linker is divinyl sulfone.

63. The water-soluble conjugate of claim 58 wherein the spacer is selected from the group consisting of: a protein, a polypeptide, bovine serum albumin, ovalbumin, or a globulin.

64. The water-soluble conjugate of claim 63 wherein the spacer is bovine serum albumin.

65. The water-soluble conjugate of claim 58 wherein the signal component is a non-particulate label.

66. The water-soluble conjugate of claim 65 wherein the non-particulate label is a dye.

67. The water-soluble conjugate of claim 66 wherein the dye is rhodamine.

68. The water-soluble conjugate of claim 58 wherein the signal component comprises a donor dye and an acceptor dye.

69. The water-soluble conjugate of claim 68, wherein the donor dye and the acceptor dye perform fluorescent resonance energy transfer (FRET) upon excitation.

70. The water-soluble conjugate of claim 59 wherein

the spacer and the non-specific protein are independently and covalently attached to the carrier via the linker;

the signal component is covalently attached to the spacer; and

the ligand to be detected or targeting element for a ligand to be detected is covalently attached to the carrier.

71. The water-soluble conjugate of claim 70 wherein the targeting element for a ligand is selected from the group consisting of: a monoclonal or polyclonal antibody, a receptor molecule, a lectin, avidin, streptavidin, and biotin.

72. The water-soluble conjugate of claim 70 wherein the ligand is selected from the group consisting of: an antigen, a hapten, a gene probe, an oligo- or polynucleotide, a mono-, oligo- or polysaccharide, a hormone, a pharmacological agent, a peptide, a lectin, avidin, streptavidin, biotin, a growth factor, a protein, or mixtures thereof.

73. The water-soluble conjugate of claim 70 wherein the ligand is selected from the group consisting of: a bacterial or viral antigen, human chorionic gonadotropin (hCG), luteinizing hormone (LH), or a drug of abuse.

74. A method for preparing a water-soluble conjugate, comprising:

a) contacting a water-soluble intermediate conjugate comprising

a carrier;

a linker;

a spacer; and

a signal component,

with i) a targeting element for a ligand to be detected, or ii) a ligand to be detected, to form a suspension containing a precipitate comprising the water-soluble conjugate; and

extracting the water-soluble conjugate from the suspension;

wherein the targeting element for a ligand to be detected or the ligand to be detected is pre-treated with a reducing agent prior to contact with the water-soluble intermediate conjugate.

75. The method of claim 74 wherein the water-soluble conjugate further comprises a spacer.

76. The method of claim 75 wherein the carrier is covalently attached to the linker and the signal component is covalently attached to the spacer.

77. The method of claim 75, wherein the preparation comprises contacting a water-soluble intermediate conjugate with the ligand to be detected or the targeting element for a ligand in the presence of a lyotropic salt at a concentration of at least about 1.25 M, and wherein the reducing agent is dithiothreitol.

78. The method of claim 77, wherein the lyotropic salt comprises components selected from the group consisting of: a sulphate, a phosphate, a citrate or tartrate of lithium, sodium, potassium, calcium and ammonium.

79. The method of claim 78 wherein lyotropic salt is present at a concentration of about 2.5 M and the salt is selected from the group consisting of: potassium phosphate and sodium phosphate.

80. The method of claim 75 further comprising that a suspension of the precipitate is subjected to a sonic frequency.

81. The method of claim 80 wherein the sonication is performed at a power of about 700 watts and a frequency of from 20 to 24 kHz.

82. The method of claim 75, further comprising that the water-soluble conjugate is separated from the supernatant by centrifugation.

83. The method of claim 75, further comprising that the water-soluble conjugate is purified from the supernatant by gel filtration.

84. The method of claim 75, wherein the carrier is selected from the group consisting of: dextran, starch, glycogen, agarose, cellulose, natural gum, and any mixture thereof.

85. The method of claim 84 wherein the carrier is dextran.

86. The method of claim 85 wherein the linker is divinyl sulfone.

87. The method of claim 86 wherein the spacer is selected from the group consisting of: a polypeptide, bovine serum albumin, ovalbumin, and a globulin.

88. The method of claim 86 wherein the spacer is bovine serum albumin.

89. The method of claim 75 wherein the signal component is a non-particulate label.

90. The method of claim 89 wherein the non-particulate label is a visual dye.

91. The method of claim 90 wherein the dye is rhodamine.

92. The method of claim 75 wherein the signal component comprises a donor dye and an acceptor dye.

93. The method of claim 92 wherein the donor dye and the acceptor dye perform fluorescent resonance energy transfer (FRET) upon excitation.

94. The method of claim 75 wherein

the carrier is covalently attached to the spacer via the linker;

the signal component is covalently attached to the spacer; and

the ligand or targeting element for a ligand is covalently attached to the carrier.

95. The method of claim 94 wherein the targeting element for a ligand is selected from the group consisting of: a monoclonal or polyclonal antibody, a receptor molecule, a lectin, avidin, streptavidin, and biotin.

96. The method of claim 94 wherein the ligand is selected from the group consisting of: an antigen, a hapten, a gene probe, an oligo- or polynucleotide, a mono-, oligo- or polysaccharide, a hormone, a pharmacological agent, a peptide, a lectin, avidin, streptavidin, biotin, a growth factor, a protein, or any mixture thereof.

97. The method of claim 96 wherein the ligand is selected from the group consisting of: a bacterial or viral antigen, human chorionic gonadotropin (hCG), luteinizing hormone (LH), or a drug of abuse.

98. The method of claim 94 wherein the targeting element is an antibody that has been treated with dithiothreitol.

99. The method of claim 96 wherein the water-soluble conjugate further comprises a non-specific protein attached to the carrier via the linker.

100. The method of claim 99 wherein the non-specific protein is an immunoglobulin or bovine serum albumin.

101. The method of claim 100 wherein the non-specific protein is covalently attached to the carrier via the linker.

102. A method for preparing a water-soluble conjugate, comprising:

contacting a water-soluble intermediate precursor comprising a carrier and a linker, with a targeting element for a ligand to be detected or a ligand to be detected, and a non-specific protein,

forming a suspension containing a precipitate of the water-soluble conjugate; and

extracting the water-soluble conjugate from the suspension.

103. The method of claim 102 wherein the water-soluble intermediate precursor further comprises a spacer component and a signal component.

104. The method of claim 103 wherein the non-specific protein is selected from the group consisting of: bovine serum albumin, an immunoglobulin, and keyhole limpet hemocyanin.

105. The method of claim 104 wherein the carrier is covalently attached to the linker and the signal component is covalently attached to the spacer, and the reducing agent is dithiothreitol.

106. The method of claim 104, wherein the water soluble intermediate conjugate is contacted with the targeting element for a ligand to be detected or ligand to be detected and non-specific protein in the presence of a lyotropic salt at a concentration of at least about 1.25 M.

107. The method of claim 106, wherein the lyotropic salt comprises components selected from the group consisting of: a sulphate, a phosphate, a citrate or tartrate of lithium, sodium, potassium, calcium and ammonium.

108. The method of claim 107 wherein lyotropic salt is present at a concentration of about 2.5 M and the salt is selected from the group consisting of: potassium phosphate and sodium phosphate.

109. The method of claim 104 wherein the water-soluble conjugate is extracted by centrifugation.

110. The method of claim 108 further comprising applying the water-soluble conjugate to a gel filtration column after extraction.

111. The method of claim 108 wherein the carrier is selected from the group consisting of: dextran, starch, glycogen, agarose, cellulose, natural gum, and mixtures thereof.

112. The method of claim 111 wherein the carrier is dextran.

113. The method of claim 104 wherein the linker is divinyl sulfone.

114. The method of claim 104 wherein the spacer is selected from the group consisting of: a polypeptide, bovine serum albumin, ovalbumin, and a globulin.

115. The method of claim 114 wherein the spacer is bovine serum albumin.

116. The method of claim 104 wherein the signal component is a dye other than a particulate dye.

117. The method of claim 104 wherein the dye is a visual dye.

118. The method of claim 117 wherein the dye is rhodamine.

119. The method of claim 104 wherein the signal component comprises a donor dye and an acceptor dye.

120. The method of claim 119 wherein the donor dye and the acceptor dye perform fluorescent resonance energy transfer (FRET) upon excitation.

121. The method of claim 104 wherein

the spacer and the non-specific protein are independently and covalently attached to the carrier via the linker;

the signal component is a dye covalently attached to the spacer;

the ligand or targeting element for a ligand is covalently attached to the carrier.

122. The method of claim 121 further comprising that

the carrier is dextran;

the linker is divinylsulfone; and

the spacer is bovine serum albumin.

123. The method of claim 104 wherein the targeting element for a ligand is selected from the group consisting of: a monoclonal or polyclonal antibody, a receptor molecule, a lectin, avidin, streptavidin, and biotin.

124. The method of claim 123 wherein the ligand is selected from the group consisting of: an antigen, a hapten, a gene probe, an oligo- or polynucleotide, a mono-, oligo- or polysaccharide, a hormone, a pharmacological agent, a peptide, a lectin, avidin, streptavidin, biotin, growth factors, a protein, an any mixture thereof.

125. The method of claim 124 wherein the ligand is selected from the group consisting of: a bacterial or viral antigen, human chorionic gonadotropin (hCG), luteinizing hormone (LH), and a drug of abuse.

126. A device comprising:

a) a water-soluble conjugate comprising:

a carrier,

a linker covalently bound to the carrier,

a signal component;

a first targeting element for a ligand to be detected or a ligand to be detected; and

a non-specific protein covalently bound to the carrier via the linker; comprised on

b) a test strip comprising a porous carrier material having i) a sample zone; ii) a detection zone wherein a liquid sample applied to the sample zone flows to the detection zone; and iii) a second targeting element for a ligand suspected to be present in the liquid sample or for a targeting element bound to the ligand, applied in the detection zone of the test strip.

127. The device of claim 126, wherein the water-soluble conjugate is movably comprised in dry form on the test strip, upstream of the detection zone and downstream of the sample zone.

128. The device of claim 126, wherein the test strip further comprises a control zone.

129. The device of claim 128, wherein the control zone is downstream from the detection zone.

130. The device of claim 126, further comprising a casing enveloping the test strip and defining the sample zone.

131. The device of claim 130, wherein the porous carrier is backed with a moisture-impervious material, the material being in contact with the inside of the casing.

132. The device of claim 130, further comprising a cap being selectively received over one end of the casing and covering the sample zone of the device.

133. The device of claim 132, wherein the casing is plastic.

134. The device of claim 126, wherein the test strip further comprises a filter situated upstream from the detection zone.

135. The device of claim 134, wherein the filter comprises a portion of the porous carrier material.

136. The device of claim 126, wherein a portion of the binding sites within the test strip are blocked with a blocking protein or polyvinyl alcohol.

137. The device of claim 136, wherein the blocking protein is bovine serum albumin or milk protein.

138. The device of claim 126, wherein the porous carrier is nitrocellulose.

139. The device of claim 126, wherein the sample is urine or serum.

140. A method for determining the presence an analyte in a liquid sample, comprising:

a) contacting a liquid sample to a portion of the test device of claim 126, the portion being located upstream from the detection zone;

b) allowing the liquid sample to flow to the detection zone; and

c) determining the presence, absence, or amount of the analyte in the liquid sample by observing the detection zone.

141. The method of claim 140, wherein step c) comprises visually observing the signal in the detection zone.

142. The method of claim 140, wherein the liquid sample is a biological sample.

143. The method of claim 144, wherein the biological sample is urine, serum, plasma, blood, semen, or mixtures thereof.

144. A method for preparing a water-soluble conjugate, comprising:

a) contacting a water-soluble intermediate precursor comprising

a carrier;

a linker;

a spacer; and

a targeting element for a ligand to be detected, or a ligand to be detected,

with i) a signal component to form a suspension containing a precipitate comprising the water-soluble conjugate; and

extracting the water-soluble conjugate from the suspension.

145. The method of claim 144 wherein the carrier is covalently attached to the linker and the signal component is covalently attached to the spacer.

146. The method of claim 144, wherein the water soluble conjugate is prepared by contacting a water-soluble intermediate conjugate with the ligand to be detected or the targeting element for a ligand in the presence of a lyotropic salt at a concentration of at least about 1.25 M.

147. The method of claim 146, wherein the lyotropic salt comprises components selected from the group consisting of: a sulphate, a phosphate, a citrate or tartrate of lithium, sodium, potassium, calcium and ammonium.

148. The method of claim 147 wherein lyotropic salt is present at a concentration of about 2.5 M and the salt is selected from the group consisting of: potassium phosphate and sodium phosphate.

149. The method of claim 144, wherein the carrier is selected from the group consisting of: dextran, starch, glycogen, agarose, cellulose, natural gum, and any mixture thereof.

150. The method of claim 149 wherein the carrier is dextran.

151. The method of claim 149 wherein the linker is divinyl sulfone.

152. The method of claim 149 wherein the spacer is selected from the group consisting of: a polypeptide, bovine serum albumin, ovalbumin, and a globulin.

153. The method of claim 152 wherein the spacer is bovine serum albumin.

154. The method of claim 144 wherein the signal component is a visual dye.

155. The method of claim 154 wherein the dye is rhodamine.

156. The method of claim 144 wherein the signal component comprises a donor dye and an acceptor dye.

157. The method of claim 144 wherein

the spacer is covalently attached to the carrier via the linker;

the targeting element for a ligand to be detected or ligand to be detected is covalently attached to the carrier via the linker.

158. The method of claim 157 wherein the targeting element for a ligand is selected from the group consisting of: a monoclonal or polyclonal antibody, a receptor molecule, a lectin, avidin, streptavidin, and biotin.

159. The method of claim 157 wherein the ligand is selected from the group consisting of: an antigen, a hapten, a gene probe, an oligo- or polynucleotide, a mono-, oligo- or polysaccharide, a hormone, a pharmacological agent, a peptide, a lectin, avidin, streptavidin, biotin, a growth factor, a protein, or any mixture thereof.

160. The method of claim 157 wherein the ligand is selected from the group consisting of: a bacterial or viral antigen, human chorionic gonadotropin (hCG), luteinizing hormone (LH), or a drug of abuse.