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(54) Titre : COMPOSITION POUR L'ADMINISTRATION D'OXYDE NITRIQUE A LA PEAU
(54) Title: COMPOSITION FOR DELIVERING NITRIC OXIDE TO SKIN

(57) Abrégé/Abstract:
A skin application composition comprising a first component in dry condition comprising a source of nitrite and a thiol and a second component comprising a source of water is provided.

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Abstract:

A skin application composition comprising a first component in dry condition comprising a source of nitrite and a thiol and a second component comprising a source of water is provided.

Composition for Delivering Nitric Oxide to Skin

Field of the Invention

The invention relates to a treatment composition, e.g. skin dressings, for application to a part of a human or animal body (for therapeutic or cosmetic purposes).

Background and prior art

Nitric oxide is an essential signalling molecule in mammals and it is known to play a variety of roles, ranging from regulation of blood flow, neurotransmission and immune response. It is so important that a family of special enzymes, called nitric oxide synthases has evolved with the exclusive job of making controlled amounts of nitric oxide when and where it is needed, using the amino acid arginine as the precursor substance.

Nitric oxide is however a very hydrophobic compound and its solubility in water is therefore limited. Maximum solubility in water achievable under normal conditions is approximately 1.7 mM, with the solubility being similar to that of oxygen.

Nitric oxide also reacts rapidly with oxygen to form nitrogen dioxide. Nitrogen dioxide has no known role in maintaining or controlling homeostasis, or ability to respond to important stimuli in biological systems. In fact, nitrogen dioxide is known as a toxin and irritant.

In addition, the biology of mammals (and other vertebrates) is capable of safely managing the logistics of nitric oxide because of the abundance of thiol groups within the tissues, especially the skin. Nitric oxide spontaneously reacts with thiol groups (e.g. on proteins) to form S-nitrosothiol functional groups, in which form the nitric oxide can be safely and efficiently stored or transported. S-Nitrosothiols are compounds capable of releasing nitric oxide. Therefore nitric oxide can also be readily released on demand via the degradation of S-nitrosothiols.

Thus, mammalian biology deals with these problems of nitric oxide, when locked up as S-nitrosothiols it can't be oxidised to nitrogen dioxide and its insolubility in water is not a problem.

However, delivery of exogenous raw nitric oxide to a skin site can result in unacceptable losses to the production of nitrogen dioxide. Also, any nitric oxide that gets into the skin can become locked into keratin S-nitrosothiols in the wrong place, or the nitric oxide can't dissolve in the available water of the system being targeted.

Various strategies therefore need to be employed before the effective delivery of the valuable nitric oxide to a skin site can be considered.

US 6,103,275 discloses a biocompatible system for generating nitric oxide by bringing together a nitrite, a reductant and a particular acid. The nitrite and acid are typically kept separate until the moment of use.

As described, S-nitrosothiols can release free nitric oxide by spontaneous decomposition, and are therefore a very convenient delivery means for the reactive and insoluble nitric oxide. However, nitrosothiols spontaneously decompose and therefore have a limited lifetime as a delivery vehicle for nitric oxide.

WO 2006/095193 discloses a skin dressing in an inactive state, but can be made to become active and deliver S-nitrosothiols to a skin site. The dressing is kept inactive either by containing reactants for the nitrosothiols so that they form the nitrosothiols only immediately before use, or by keeping pre-formed nitrosothiols in a dry state, to be activated when needed by addition of water.

The rate of decomposition varies considerably depending on the side chain of the thiol. For example, whilst nitrosocysteine can be totally decomposed within minutes under normal conditions, it takes hours/days to achieve 100% decomposition of nitrosoglutathione. The decomposition is generally accelerated in the presence of Cu^{2+} and Hg^{2+}.

Summary of the Invention

The present invention mimics the natural biological process by directly synthesising S-nitrosothiols which can safely escort nitric oxide into the target location, without forming nitrogen dioxide, and in a water-soluble form. By their chemical nature, the S-nitrosothiols can readily exchange their nitric oxide with thiols of the body, thereby efficiently interposing the delivered nitric oxide into the body, harmonising with the nitric oxide logistics of the body.

Thus, the present invention provides a skin application composition comprising a first component in dry condition comprising a source of nitrite and a thiol and a second component comprising a source of water.

As the nitrite and thiol are in dry condition the treatment composition is in an inactive state. However, the treatment composition can be activated, by bringing the first and second components into contact with each other, allowing the nitrite and thiol to react to form S-nitrosothiol immediately prior to use, to allow the active delivery of the S-nitrosothiols to the skin site to be treated.

Dry condition means that there is no free water in the first component, such that no significant or measurable water loss occurs through evaporation under normal ambient conditions of temperature, pressure and humidity. Dry condition includes desiccated condition, which is an extra thoroughly dried condition. Desiccated condition means a condition maintained by storage in an environment enclosed by a moisture impermeable barrier, wherein the material is kept scrupulously free of water by means of an added desiccant.

Elevated nitric oxide can have beneficial effects on tissues suffering from inadequate blood perfusion, through its vasodilatory effect which causes blood capillaries in the vicinity to open up leading to improved blood circulation. The vasodilatory effect can also enhance transdermal delivery of materials such as a pharmaceutically active agent, e.g. hormones, analgesics etc, by accelerating delivery and uptake of the materials. The composition can thus also be used as an adjuvant for transdermal delivery, typically by having a composite dressing or patch, plaster, bandage, gauze etc. also including material for delivery.

Preferably the skin treatment composition is a skin dressing. The term "skin dressing" covers dressings such as patches, plasters, bandages, absorbent foams and gauze etc.. The term also includes material in amorphous or liquid form. The term covers dressings for application to body surfaces generally, including internal and external tissues, particularly the skin including the scalp.

Suitable S-nitrosothiols include S-nitrosoglutathione (preferably S-nitroso-L-glutathione, as this is the physiologically important version), S-nitrosocysteine, S-nitrosothioglycerol, S-nitroso-N-acetylcysteine, S-nitrosocaptopril, S-nitrosomercaptoethylamine, S-nitroso-3-

mercaptopropanoic acid, S-nitroso-D-thioglucose and S-nitroso-N-acetyl-D, L-penicillamine. S-nitrosoglutathione is currently preferred, because of its relatively slow rate of decomposition to generate nitric oxide, resulting in satisfactory stability of the S-nitrosothiol in the dressing and consequential slow release of nitric oxide at an appropriate rate for skin benefits.

The source of nitrite is preferably a nitrite salt such as sodium nitrite or potassium nitrite, and the thiol is preferably thioglycerol, thioglucose or glutathione.

Although the nitrite and the thiol are both present in the first component, the nitrite and thiol are preferably not in intimate contact with each other. This is to ensure that in addition to being in a dried state, the lack of intimate contact maintains the treatment composition in an inactive state until desired for use.

In a preferred embodiment, the first component may be provided as a solid material with the dry nitrite and thiol provided therein. The solid material preferably comprises a polymer material.

Preferred polymers include water-soluble polymers such as polyvinyl alcohol (PVA), polyvinyl pyrrolidone, cellulose or modified cellulose (such as carboxymethylcellulose). One preferred polymer material comprises PVA. PVA has convenient and acceptable properties for skin treatment use, e.g. being non-toxic. PVA is also easy to handle and use, readily forming a film on drying of a PVA solution in water, with the resulting film being easy to handle. PVA is also readily available and cheap. Cross-linking is not required to form a solid material, e.g. in the form of a film, although cross-linking may optionally be employed. PVA is available in a wide range of grades based on molecular weight and degree of hydrolysis, which affect the physical properties of the material. Appropriate grades of PVA can be readily selected to produce a polymer product having desired properties for a particular intended use. For example, for use in skin dressings, good results have been obtained by use of PVA with a molecular weight in the range 100,000 to 200,000, substantially fully hydrolysed (98-99% hydrolysed), e.g. in the form of code 36,316-2 from Aldrich, in non-cross-linked form, and M_w 31,000-50,000, 98-99% hydrolyzed – obtained from Sigma (363138).

Another suitable polymer material comprises polyvinylpyrrolidone (PVP). The properties of PVP are very similar to those of PVA, and PVP is also acceptable for skin treatment use. PVP is readily available in a range of different molecular weights. Appropriate grades of PVP can be readily selected. For example, good results have been obtained using a PVP having a molecular weight average of 360,000, e.g. in the form of code PVP360 from Sigma, in a non-crosslinked form.

Mixtures of polymer materials may be used.

The solid material is conveniently in the form of a sheet, layer or film, typically having a thickness in the range 0.01 to 1.0mm, preferably in the range 0.05 to 0.5mm. Preferably the nitrite will be provided in one sheet, layer or film and the thiol will be provided in another sheet, layer or film. The two sheets can then be placed together to form the first component.

The solid material may optionally include a support to provide rigidity when wet.

The solid material of the invention is conveniently made by mixing a solution of a polymer (e.g. an aqueous solution of PVA and/or PVP) and reagent, and drying the mixture to produce a solid material, e.g. forming film by a casting procedure. Suitable techniques are well known to those skilled in the art.

The polymer material or materials are suitably used in appropriate amounts that result in formation of a film, with the upper limit of concentration typically being dictated by the limit of solubility (generally in water) and the lower limit of concentration being the point at which a film does not form. For PVA code 36,316-2 from Aldrich, the limit of solubility in water is about 6% w/w, resulting in a concentration of PVA in the film prior to drying of about 5%.

Such solid polymer materials are typically in dry condition, and therefore can be used on exuding wounds, which may provide the source of water. However, preferably such solid polymer materials will be provided with a second component comprising a source of water, as discussed below, and can therefore also be used on dry wounds. Solid polymer materials are also particularly suitable for burns and similar skin conditions.

Alternatively, the first component may be provided in the form of a porous water-absorbable material such as a mesh or foam, onto which the nitrite or thiol is provided in dried form.

Such a mesh or foam is preferably made from a solid water-absorbent polymer, e.g. silicone, polyethylene, polypropylene polystyrene, polyurethane, polyacrylate and polyamide. Preferably the nitrite will be provided on one mesh and the thiol provided on another mesh. The two meshes can then be placed together to form the first component. Examples include the Allevyn™ range (Smith & Nephew), Biatain™ range (Coloplast), Lyofoam™ range (Molnlycke), Tielle™ range (Systagenix) and Tegaderm™ range (3M).

Alternatively the mesh or foam may be made from a material such as alginate. Such porous materials are applicable to exuding wounds. Examples include AlgisiteM™ range (Smith & Nephew), Biatain™ range (Coloplast), Kaltostat™ range (ConvaTec), Tegaderm™ range (3M) and Urgosorb™ range (Urgo).

Such meshes or foams may be prepared by applying an aqueous solution of nitrite or thiol, e.g. by dipping, spraying etc, which is allowed to be absorbed into the porous structure of the mesh or foam. This is then followed by a drying step, leaving behind nitrite or thiol in dry condition adhered to the structure of the mesh or foam.

If applied to an exuding wound, a mesh or foam can begin to absorb wound exudate to create a liquid flow pathway from the mesh or foam to the wound site. The nitrite and thiol can then dissolve into the wound exudate and generate S-nitrosothiol which in turn diffuses towards the wound site down the concentration gradient generated.

Alternatively, the first component may comprise a non-aqueous liquid matrix containing the nitrite and thiol dispersed therein. In one arrangement the nitrite and thiol may be separately dispersed in the matrix as fine particulate material, i.e. a non-aqueous liquid matrix comprising dispersed particles of thiol and dispersed particles of nitrite. In another arrangement there may be two non-aqueous liquid matrices, one for the thiol and one for the nitrite. In this case, the nitrite and thiol may be separately dispersed in their respective matrix as fine particulate material or may be dissolved into the matrix itself. Suitable non-aqueous liquids include propylene glycol, polyethylene glycol (e.g. PEG300, PEG400, PEG3350), and can include non-aqueous creams, ointments or lotions.

Such non-aqueous liquids are applicable to exuding wounds. In a similar manner to the meshes and foams, the water to enable reaction of nitrite with thiol and subsequent release of the S-nitrosothiol generated may be provided by wound exudate.

Alternatively, the first component may be provided in the form of a particulate material such as a powder or in granular form. In such an arrangement a first particulate material will comprise the nitrite and a second particulate material will comprise the thiol. The two particulate components can then be combined, and even blended together, to form the first component.

The first component may also comprise a chelating agent, capable of chelating divalent metal ions such as Cu^{2+}, Zn^{2+} and/or Fe^{2+}. Suitable chelating agents include EDTA, EGTA, histidine and/or citrate.

It is highly preferred that the nitrite is kept at a near neutral pH, such as from 4.0 to 8.0, preferably from 5.0 to 7.5, most preferably from 6.0 to 7.5. However the thiol should be kept acidic with a pH of from 1.0 to 4.0, preferably from 2.0 to 3.5. Suitable materials for maintaining these pH levels such as buffers are therefore preferably included.

The composition is activated, by bringing the first component into contact with the second component, resulting in the reaction between the nitrite and thiol and formation of one or more S-nitrosothiols. On activation of the dressing, the nitrite and the thiol come into intimate contact. Mixing of the nitrite with the thiol in acidic solution results in slow generation of S-nitrosothiol. If the thiol is L-glutathione, then the product of reaction is S-nitroso-L-glutathione. Once produced, the S-nitrosothiol is released from the dressing into the surrounding environment, e.g. into a wound bed, where it decomposes to produce nitric oxide, with consequential beneficial effects.

Thus, it is desirable that, following the bringing together of the nitrite and thiol, the mixture of the first and second components has an acidic pH of preferably less than 4.0 to facilitate the rapid generation of S-nitrosothiols. The treatment composition may therefore optionally include and/or generate on activation a source of protons.

The preferred nitric oxide donor to be generated by the activated dressing is S-nitrosoglutathione (GSNO). The rate of GSNO production in aqueous environment containing glutathione and nitrite is pH dependent. Glutathione is itself an acidic compound capable of donating protons to allow generation of GSNO in the activated dressing, so an additional source of protons is not always essential.

The additional source of protons will typically be in the form of a buffer, for example, lactate buffer, acetate buffer acid, citrate buffer, succinate buffer or citrate-phosphate buffer. Buffer may be included in one or both of the first and second components.

Incorporation of the additional source of protons allows a degree of control over the rate of S-nitrosothiol production inside the treatment composition. The rate of the production increases with the acidity of the dressing regulated by the buffer incorporated. Thus, for example, the rate of the S-nitrosothiol production will be slower if phosphate buffer (pH 5.5) is incorporated as the source of protons compared with incorporation of citrate buffer (pH 3).

The second component may be a hydrated hydrogel. A hydrated hydrogel means one or more water-based or aqueous gels, in hydrated form. A hydrated hydrogel thus includes a source of water, for activation of the treatment composition. A hydrated hydrogel can also act to absorb water and other materials exuded from a wound site, enabling the treatment composition to perform a valuable and useful function by removing such materials from a wound site. The hydrated hydrogel also provides a source of moisture that can act in use to maintain a wound site moist, aiding healing.

Suitable hydrated hydrogels are disclosed in WO 03/090800. The hydrated hydrogel conveniently comprises hydrophilic polymer material. Suitable hydrophilic polymer materials include polyacrylates and methacrylates, e.g. as supplied by First Water Ltd in the form of proprietary hydrogels, including poly 2-acrylamido-2-methylpropane sulphonic acid (poly-AMPS) and/or salts thereof (e.g. as described in WO 01/96422), polysaccharides e.g. polysaccharide gums particularly xanthan gum (e.g. available under the Trade Mark Keltrol), various sugars, polycarboxylic acids (e.g. available under the Trade Mark Gantrez AN-169 BF from ISP Europe), poly(methyl vinyl ether co-maleic anhydride) (e.g. available under the Trade Mark Gantrez AN 139, having a molecular weight in the range 20,000 to 40,000), polyvinyl pyrrolidone (e.g. in the form of commercially available grades known as PVP K-30 and PVP K-90), polyethylene oxide (e.g. available under the Trade Mark Polyox WSR-301), polyvinyl alcohol (e.g. available under the Trade Mark Elvanol), cross-linked polyacrylic polymer (e.g. available under the Trade Mark Carbopol EZ-1), celluloses and modified celluloses including hydroxypropyl cellulose (e.g. available under the Trade Mark Klucel EEF), sodium carboxymethyl cellulose (e.g. available under the Trade Mark Cellulose Gum 7LF) and hydroxyethyl cellulose (e.g. available under the Trade Mark Natrosol 250 LR).

Mixtures of hydrophilic polymer materials may be used in a gel.

In a hydrated hydrogel of hydrophilic polymer material, the hydrophilic polymer material is desirably present at a concentration of at least 1%, preferably at least 2%, more preferably at least 5%, yet more preferably at least 10%, or at least 20%, desirably at least 25% and even more desirably at least 30% by weight based on the total weight of the gel. Even higher amounts, up to about 40% by weight based on the total weight of the gel, may be used.

Good results have been obtained with use of a hydrated hydrogel of poly-AMPS and/or salts thereof in an amount of about 30% by weight of the total weight of the gel.

By using a gel comprising a relatively high concentration (at least 2% by weight) of hydrophilic polymer material, the gel can function particularly effectively to take up water in use of the treatment composition, e.g. from serum exudates while in contact with a wound. Because the gel is an aqueous system, use of the treatment composition does not have the effect of inducing an overall dryness of the wound which would be undesirable. This is because water vapour pressure is maintained in the enclosed environment surrounding the skin in use of the treatment composition. The gel thus functions as an absorbent entity for the removal of moisture, e.g. wound exudate, that also provides a helpful background level of excess moisture.

The water-uptake capacity of a hydrated hydrogel, including a high concentration gel, enables the treatment composition to aid wound healing by removing substantial amounts of exudates, swelling-up as it does so. By using a carefully formulated, ready-hydrated gel, the wound is prevented from reaching a state of unhelpful dryness. Ready hydration also ensures the quick formation of an aqueous liquid interface between the treatment composition and the wound, thus preventing adhesion, which otherwise would interfere with easy lifting of the treatment composition when it has to be replaced. A good aqueous liquid interface between the wound and the treatment composition is also important in allowing any beneficial products carried in the gel to enter the wound through all of the available surface.

The hydrated hydrogel material is typically in the form of a solid layer, sheet or film of material that is typically cross-linked, and that may incorporate a mechanical reinforcing

structure. The size and shape of the layer, sheet or film can be selected to suit the intended use of the treatment composition. Thicknesses in the range 0.05 to 5 mm, preferably 0.5 to 3 mm are particularly suitable. Examples of such gels include the ActiFormCool™ range (L&R) and Intrasite™ range (Smith & Nephew).

Alternatively, the hydrated hydrogel may be in the form of an amorphous gel not having a fixed form or shape that can be deformed and shaped in three dimensions, including being squeezed through a nozzle. Amorphous gels are typically not cross-linked or have low levels of cross-linking. A shear-thinning amorphous gel may be used. Such a gel is liquid when subjected to shear stress (e.g. when being poured or squeezed through a nozzle) but set when static. Thus the gel may be in the form of a pourable or squeezable component that may be dispensed, e.g. from a compressible tube or a syringe-like dispenser, comprising a piston and cylinder, typically with a nozzle of about 3 mm diameter. Such a gel may be applied in the form of a surface layer, or into a wound cavity as a fully conformable gel that fills the available space and contacts the wound surface. Examples of such gels include the Purilon™ range (Coloplast), Nu-Gel™ range (Systagenix), Granugel™ range (ConvaTec) and Intrasite™ range (Smith and Nephew).

A typical example of an amorphous gel formulation is: 15% w/w AMPS (sodium salt), 0.19% polyethylene glycol diacrylate and 0.01% hydroxycyclohexyl phenyl ketone, with the volume made up to 100% with analytical grade DI water. The reagents are thoroughly mixed and dissolved, then polymerised for between 30-60 seconds, using a UV-A lamp delivering approximately 100 mW/cm^2, to form the required hydrogel. This may be contained in plastic syringes from which the amorphous gel may then be dispensed from a syringe to a target site, as a surface layer or to fill a cavity.

The second component may alternatively comprise a hydro-cream carried in a suitable container such as a tub or a squeezable pouch or tube.

Thus, in one preferred embodiment the invention comprises a first component comprising two layers of dry polymeric matrix, preferably dried PVA, containing the nitrite and thiol respectively and a second component comprising a layer of hydrated hydrogel. The second component may be used in contact with the skin, as the hydrated hydrogel has beneficial properties for skin contact, as discussed above, with the first component being placed on top of the second component. Provided the components are kept separate prior to use, the

treatment composition remains in non-activated condition. However, when the two components are brought into contact, this has the effect of activating the treatment composition.

In another preferred embodiment, the treatment composition comprises components which are amorphous. This is particularly the case for the preferred embodiment where the S-nitrosothiol is dispersed in a non-aqueous liquid matrix. The amorphous components can be in the form of e.g. a gel, semi-solid, paste, cream, lotion or liquid. Such an amorphous component may be provided on its own and derive the needed water from the wound exudate itself. Alternatively water may be provided by hydrated hydrogels or other amorphous material, as discussed above.

The two amorphous components are kept separate until it is desired to apply the treatment composition to a body surface. Conveniently they are packaged in a container having a nozzle, through which the amorphous components can be delivered. Preferably, the two components are packaged in a two compartment dispenser, preferably being operable to deliver both components simultaneously.

The treatment composition optionally includes, or is used with, a covering or outer layer for adhering a dressing to the skin of a human or animal in known manner.

Treatment compositions in accordance with the invention can be manufactured in a range of different sizes and shapes for treatment of areas of skin e.g. wounds of different sizes and shapes. Appropriate amounts of reagents for a particular dressing can be readily determined by experiment.

Treatment composition components are suitably stored prior to use in sterile, sealed, water-impervious packages, e.g. laminated aluminium foil packages. To ensure a dry condition is maintained, desiccant material is desirably included in the package for the first component.

In use, the treatment composition component or components are removed from their packaging and brought into contact, e.g. by being located in appropriate order on the skin of a human or animal, e.g. over a wound or other region of skin to be treated for cosmetic or therapeutic purposes. The treatment composition may also be used as an adjuvant for transdermal delivery, as noted above.

Examples

A number of model systems were prepared to determine the production and generation rate of S-nitrosothiols. The systems are detailed in tables at the start of each results section below. For each system, sample aliquots from the aqueous component were taken, at three time points after the system 'activation' (i.e. all components being brought together) to confirm the presence of S-nitrosothiol generation and release. The first time-point was always t=zero (i.e. measuring S-nitrosothiol in the aqueous component prior to being 'activated' with the dry/non-aqueous component to demonstrate there was no S-nitrosothiol at the start, which was the case in all systems), then t=2 hours after activation and finally t=6hours after activation.

To demonstrate that the S-nitrosothiols are being generated by reaction between the nitrite and thiol, systems were prepared where (1) the nitrite and thiol are kept separate prior to use and (2) the nitrite and thiol are mixed together during manufacture together and pre-generate S-nitrosothiols prior to use.

Materials

- Sodium nitrite (300 mM) in DI water
- Glutathione (300 mM) in DI water
- Thioglycerol (300 mM) in DI water
- Lactic acid (100 mM) in DI water – (adjusted to pH 4.0 with 0.2M NaOH)
- Sorbitol (1 M) in DI water
- Polyvinyl alcohol (7.5% w/w) in DI water – M_w 31,000-50,000, 98-99% hydrolyzed – obtained from Sigma (363138)EDTA (disodium) (5 mM) in DI water
- Copper (2+) Nitrite (5 mM) in DI water

PVA Stock Solution Manufacture Procedure

462.5ml of DI water was measured out and heated on hot plate to constant temperature between 80-85°C - controlled with digital thermometer. 37.5g PVA powder was measured out and divided onto 5 x 7.5g aliquots. Single aliquots of the PVA powder were added to the heated water which was being stirred (preventing PVA coagulation). Throughout the

additions, the water/PVA temperature was maintained at 80-85°C). Additions were repeated while maintaining the temperature of the water/PVA mix until the PVA is dissolved. After removal from the hotplate and cooling, the final volume was made up to 500ml with DI water.

PVA Films Manufacture

PVA films were produced by mixing the PVA stock solution with active components and allowing the mixture to dry in a Petri plate at 40°C. 20 ml of each pre-prepared PVA solution comprising the active components was poured into 10 × 10 cm Petri plates and left to dry overnight in an incubator at 40°C. The PVA films were produced with the active components in two separate films to be brought together each comprising a nitrite or a thiol (PVA1, PVA2 and PVA3) or the nitrite and thiol included together in a single film (PVA4 and PVA5). In both cases the formed films are to be brought together with an aqueous system to activate release of S-nitrosothiols. The composition of the PVA mixtures prior to drying is shown in Table 1.

In the case of PVA4 and PVA5 it is assumed that the nitrite and thiol react together to form S-nitrosothiol during manufacture and prior to formation of the PVA film, and are included as comparative examples.

Table 1

Film ID	Film Components
PVA1	PVA (5% w/w) Sodium nitrite (30 mM)
PVA2	PVA (5% w/w) Glutathione (30 mM) Lactic acid (5 mM) at pH 4.0
PVA3	PVA (5% w/w) Thioglycerol (30 mM) Lactic acid (5 mM) at pH 4.0
PVA4	PVA (5% w/w) Glutathione (30 mM) Sodium nitrite (30 mM) Lactic acid (5 mM) at pH 4.0
PVA5	PVA (5% w/w) Glutathione (30 mM) Sodium nitrite (30 mM) Lactic acid (5 mM) EDTA (0.05 mM) at pH 4.0

Powder Manufacture

Powders comprising nitrite or thiol were produced by mixing the nitrite or the thiol with a bulking agent (sorbitol), followed by drying the mixture. For each powder, 20 ml of each pre-prepared solution was poured into 10 x 10 cm Petri plates and left to dehydrate for 24 hours in an incubator at 40°C, followed by a thorough desiccation. Once in powder form, the formulations were dispersed in neat Propylene Glycol (0.1 g of powder to 1ml Propylene Glycol).

The composition of the aqueous mixtures for powder preparation prior to drying is shown in Table 2.

Powders were activated for S-nitrosothiol release, once brought into contact with an aqueous system (such as a hydrogel). Powders were produced with the active components,

in two separate powders to be brought together each comprising a nitrite or a thiol (P1 and P2) or the nitrite and thiol included together in a single powder (P3 and P4).

In the case of P3 and P4 it is assumed that the nitrite and thiol react together to form S-nitrosothiol during manufacture and prior to formation of the powder and are included as comparative examples.

Table 2

Powder ID	Powder Components
P1	Sorbitol (500 mM) Sodium nitrite (30 mM)
P2	Sorbitol (500 mM) Glutathione (30 mM) Lactic acid (5 mM) at pH 4.0
P3	Sorbitol (500 mM) Glutathione (30 mM) Sodium nitrite (30 mM) Lactic acid (5 mM) at pH 4.0
P4	Sorbitol (500 mM) Glutathione (30 mM) Sodium nitrite (30 mM) Lactic acid (5 mM) EDTA (0.05 mM) at pH 4.0

Aqueous Components

The pre-prepared PVA films and Powders require contact with an aqueous component to activate S-nitrosothiol generation and release. The various aqueous components are shown below in table 3.

Table 3

AQ ID	Aqueous Component
AQ1	Sheet hydrogel
AQ2	Sheet hydrogel imbibed with 5mM Copper Nitrite Solution (50µL of $Cu(NO_3)_2$ per $1cm^2$ hydrogel)
AQ3	Amorphous Hydrogel
AQ4	Amorphous Hydrogel (50µL of 5mM $Cu(NO_3)_2$ per 1cm2 hydrogel)
AQ5	DI water
AQ6	0.2mM $Cu(NO_3)_2$ Solution (in DI water)

Details of the sheet hydrogel and amorphous hydrogel materials are shown in Table 4.

Table 4

Component	Name	Manufacturer	Details
Sheet hydrogel	ActiformCool	Activa	70% H_2O: 30% Acrylic Polymer (Taurate derivative). Phenoxyethanol as preservative. Used in moderate to heavily exuding wounds
Amorphous Hydrogel	ActivHeal	Advanced Medical Solutions	Hydrogel with high water content – 85%. Used in nil to low exudate wounds
Foam	ActivHeal	Advanced Medical Solutions	Non-adhesive absorbent Polyurethane. Used in moderate to heavily exuding wounds. Total Fluid Handling ca. $24g/10cm^2$ [1]

S-Nitrosothiol Measurement

The presence of generated S-nitrosothiols was measured by an Absorbance reading at 490nm using the Griess reagent method described below. S-nitrosothiol concentration can be calculated from the absorbance measurement using the extinction coefficient of ca. 10,000 M^{-1} cm^{-1}. Absorbance measurement was carried out using Fisherbrand™ Digital Colorimeter Model 45.

Two different methods are required to calculate generated S-Nitrosothiol concentrations depending on whether a Hydrogel (AQ1 to AQ4) or a Solution (AQ5 and AQ6) are utilised as the aqueous component.

Reagents for S-nitrosothiol measurement

Reagent 1:	Na-phosphate buffer (pH 7.4, 0.1 M).
Reagent 2:	Griess reagent: 20 mg of N-(1-Naphthyl)ethylendiamine dihydrochloride (NADD) + 500 mg of sulphanilamide dissolved in 2 mL of DMSO.
Reagent 3:	Mercuric chloride (10 mM) in DMSO (13.58 mg of HgCl2 in 5 mL of DMSO).

Procedure to measure S-nitrosothiol concentration in gels

1. Dispense 25 mL of Reagent 1 and 825 µL of Reagent 2 into a 250 ml pot
2. Weigh accurately 300 mg of the hydrogel and immerse it in the reagent mix. Incubate while shaking mildly for 30 min.
3. Transfer 2.6 ml of the reagent mix from the pot into a plastic cuvette
4. Add 25 µl of Reagent 3
5. Read absorbance of the resulting mixture at 490 nm in 10 min

Procedure to measure S-nitrosothiol concentration in solutions

1. Dispense 1.5 mL of Reagent 1 into a plastic cuvette
2. Add 200 µL of the sample
3. Add 1.17 µL of DI water

4. Add 100 µL of Reagent 2
5. Add 30 µL of Reagent 3 and mix thoroughly
6. Read absorbance of the resulting mixture at 490 nm in 10 min

Results

S-nitrosothiol measurements in PVA film systems

Generation and release of S-nitrosothiol from PVA film systems comprising separate sources of nitrite and thiol, following activation by contact with aqueous systems is shown in Table 5. In each case the measurements were repeated, and the results are shown in mAU.

Table 5

PVA film 1	PVA film 2	Aqueous component	Absorbance at t=0	Absorbance at 2 hrs	Absorbance at 6 hours
PVA1	PVA2	Aq1	0 0	70 60	160 140
PVA1	PVA2	Aq2	0 0	40 50	0 50
PVA1	PVA2	Aq3	0 0	50 40	110 110
PVA1	PVA2	Aq4	0 0	10 0	70 70
PVA1	PVA3	Aq1	0 0	0 0	0 0
PVA1	PVA3	Aq2	0 0	0 0	0 0
PVA1	PVA3	Aq3	0 0	0 10	30 60
PVA1	PVA3	Aq4	0 0	0 0	40 30

The following combinations, comprising pre-mixed nitrite and thiol were tested as comparative examples, as shown in table 6.

Table 6

PVA film	Aqueous component	Absorbance at t=0	Absorbance at 2 hours	Absorbance at 6 hours
PVA4	Aq1	0 0	10 10	60 50
PVA4	Aq2	0 0	0 0	60 40
PVA4	Aq3	0 0	70 70	90 80
PVA4	Aq4	0 0	20 20	40 50
PVA5	Aq1	0 0	10 0	20 20
PVA5	Aq2	0 0	20 10	30 30
PVA5	Aq3	0 0	40 40	80 90
PVA5	Aq4	0 0	20 20	100 90

S-nitrosothiol measurements in systems based on a non-aqueous liquid component

Generation and release of S-nitrosothiol from powder based non-aqueous systems comprising separate sources of nitrite and thiol, following activation by contact with aqueous systems is shown in Table 7. In each case the measurements were repeated, and the results are shown in mAU.

Table 7

Non-aqueous component 1	Non-aqueous component 2	Aqueous component	Absorbance at t=0	Absorbance at 2 hrs	Absorbance at 6 hours
P1	P2	Aq3	0 0	20 10	10 10
P1	P2	Aq4	0 0	10 10	10 10
P1	P2	Aq5	0 0	370 370	290 300
P1	P2	Aq6	0 0	450 450	420 420

The following combinations comprising pre-mixed nitrite and thiol were tested as comparative examples, as shown in table 8.

Table 8

Non-aqueous component 1	Aqueous component	Absorbance at t=0	Absorbance at 2 hrs	Absorbance at 6 hours
P3	Aq3	0 0	30 30	50 50
P3	Aq4	0 0	40 30	30 30
P3	Aq5	0 0	80 80	50 50
P3	Aq6	0 0	70 70	0 0
P4	Aq3	0 0	10 0	40 30
P4	Aq4	0 0	0 0	0 0
P4	Aq5	0 0	80 70	60 60
P4	Aq6	0 0	50 30	0 0

Conclusions

Generation and release of S-nitrosothiol from non-aqueous components based on PVA films and powder based systems was demonstrated on contact with an aqueous component.

Claims

1. A skin application composition comprising a first component in dry condition comprising a source of nitrite and a thiol and a second component comprising a source of water.

2. A skin application composition according to claim 1, wherein the application composition is a skin dressing.

3. A skin application composition according to claim 1 or claim 2, wherein the source of nitrite is a nitrite salt.

4. A skin application composition according to any one of the preceding claims, wherein the nitrite and thiol are not in intimate contact with each other.

5. A skin application composition according to any one of the preceding claims, wherein the first component is a solid material.

6. A skin application composition according to claim 5, wherein the solid material comprises a polymer material.

7. A skin application composition according to claim 6, wherein the polymer material comprises polyvinyl alcohol or polyvinyl pyrrolidone or a mixture thereof.

8. A skin application composition according to any one of claims 5 to 7, wherein the solid material is in the form of a sheet, layer or slab.

9. A skin application composition according to any one of claims 1 to 4, wherein the first component comprises a non-aqueous liquid matrix comprising the nitrite and thiol dispersed therein.

10. A skin application composition according to any one of claims 1 to 4, wherein the first component is provided in the form of particulate material.

11. A skin application composition according to any one of claims 4 to 10, wherein the nitrite is kept at a pH of from 4.0 to 8.0, preferably from 5.0 to 7.5, most preferably from 6.0 to 7.5.

12. A skin application composition according to any one of claims 4 to 11, wherein the thiol is kept at a pH of from 1.0 to 4.0, preferably from 2.0 to 3.5.

13. A skin application composition according to any one of the preceding claims, wherein bringing the first and second components together into intimate contact results in a pH of less than 4.0.