

Description

Title of Invention: Oxidant-Dependent Time Indicator

Technical Field

The present invention relates to microfluidic indicators and in particular to oxidant-dependent indicators.

Background Art

Oxygen indicators are widely used in modified atmosphere packaging (MAP) to prolong the useful life of many oxygen-sensitive items, such as: food, beverages, works of art, pharmaceuticals, medical diagnostic kits and sterilised packages. A number of indicators for this purpose, such as colorimetric indicators, have been reported previously.

Most colorimetric oxygen indicators comprise a redox-sensitive dyestuff, an alkaline substance and a reducing agent. In an anaerobic atmosphere, the reducing agent reduces the usually highly-coloured oxidized form of the redox-sensitive dye to its reduced, usually lesser-coloured form. Usually, the reduced form is readily oxidized back to the oxidized form in an aerobic atmosphere. Accordingly, the presence of oxygen is easily determined by its color.

However, current colorimetric oxygen indicators have a number of problems that have prevented their extensive application in MAP. These problems include one or more of the following: a relatively high cost, short shelf-life and a response that is affected by the presence of carbon dioxide (a common MAP packaging gas). The high cost typically comes from the specific atmospheres, such as low-oxygen or nitrogen atmospheres, or ultraviolet irradiation required to produce and use the indicator.

In addition, colorimetric oxygen indicators usually give a reversible response towards oxygen. However, the latter is not a desirable feature in, e.g. MAPed foods, since microbial growth can be so rapid that oxygen leaking into the package is metabolized after a short time if the integrity of an oxygen-free MAPed food package is compromised and air is let in. This is particularly true if the air leak is small and goes undetected by the oxygen indicator despite the compromise in the integrity of the package.

It is known that non-reducing acid accelerates the reduction of methylene blue. An oxygen indicator coated or supported on a substrate is also known. However, such indicators comprise methylene blue mixed with polymeric matrices or thickeners as oxygen barriers to control diffusion of oxygen and initiate the redox reactions.

Further, different reaction response times require different types of chemical additives. Also, nitrogen atmospheres or ultraviolet irradiation are required to produce and use the indicator.

Accordingly, there is a need to provide an alternative indicator that overcomes, or at least ameliorates, one or more of the disadvantages described above.

Summary

There is provided an irreversible, colorimetric, oxidant-dependent time indicator comprising: a) a porous material substrate; b) one or more redox-active dyes; c) one or more reductants; d) one or more non-alkaline additives; characterized in that the components b) to c) are deposited on the substrate a) substantially in the absence of a polymeric material or thickener in the component matrix and substantially in the absence of an oxidant semipermeable coating layer on the substrate.

According to a first aspect, there is provided an irreversible, colorimetric, oxygen-dependent time indicator comprising: a) a porous material substrate; b) one or more redox-active dyes; c) one or more reductants; d) one or more non-alkaline additives; characterized in that the components b) to c) are deposited on the substrate a) substantially in the absence of a polymeric material or thickener in the component matrix and substantially in the absence of an oxygen semipermeable coating layer on the substrate at the time of deposition, wherein at least one reductant is selected from the group consisting of ascorbic acid, erythorbic acid, enantiomers of ascorbic acid and erythorbic acid, hydrophilic phosphine and combinations thereof, wherein the at least one non-alkaline additive is an acidic additive selected from water-soluble strong acids.

There is provided a method of using an indicator as disclosed herein to measure the oxidant exposure time wherein the indicator is activated by exposure to a constant oxidant concentration in its environment and its irreversible time dependent change in colour is observed after activation.

According to a second aspect, there is provided a method of using an indicator as disclosed herein to measure the oxygen exposure time wherein the indicator is activated by exposure to a constant oxygen concentration in its environment and its irreversible time dependent change in colour is observed after activation.

According to a third aspect, there is provided a process for making an indicator as disclosed herein, characterized in that a solution of the reductants and additives is added to a solution of a redox-active dye and deposited on the porous material substrate; or solution of the reductants and additives is added to a pre-dried spot of a redox-active dye solution on the porous material substrate.

There is provided an oxidant exposure time measuring device comprising one or more indicators as disclosed herein.

According to a fourth aspect, there is provided an oxygen exposure time measuring device comprising one or more indicators as disclosed herein.

According to a fifth aspect, there is provided a two or multilayer device according to the fourth aspect, wherein an indicator on one layer of porous material as disclosed herein is covered with another layer of porous material that contains further additives.

There is provided a kit of parts comprising a device as disclosed herein together with an oxidant impermeable container wherein the device is stored.

According to a sixth aspect, there is provided a kit of parts comprising a device as disclosed herein together with an oxygen impermeable container wherein the device is stored.

Definitions

The following words and terms used herein shall have the meaning indicated:

The term “dye” refers to substances which impart color to a substrate by selective absorption of light. Dyes are retained in the substrate by absorption, solution, mechanical retention, or by ionic or covalent chemical bonds.

The term “redox” refers to a chemical reaction involving a reduction (accepting of an electron(s)) and a corresponding oxidation (donation of an electron(s)). Accordingly, the term “redox-active dye” refers to a dye that is capable of being oxidized or reduced. The term “redox-sensitive” is used interchangeably herein.

The term “oxidant” refers to either a chemical compound that readily transfers oxygen atoms and oxidize other compounds, or a substance that gains electrons in a redox chemical reaction.

The term “ambient” is to be broadly interpreted as the surrounding area or environment.

The word “substantially” does not exclude “completely” e.g. a composition which is “substantially free” from Y may be completely free from Y. Where necessary, the word “substantially” may be omitted from the definition of the invention.

Unless specified otherwise, the terms "comprising" and "comprise", and grammatical variants thereof, are intended to represent "open" or "inclusive" language such that they include recited elements but also permit inclusion of additional, unrecited elements.

As used herein, the term "about", in the context of concentrations of components of the formulations, typically means +/- 5% of the stated value, more typically +/- 4% of the stated value, more typically +/- 3% of the stated value, more typically, +/- 2% of the stated value, even more typically +/- 1% of the stated value, and even more typically +/- 0.5% of the stated value.

Throughout this disclosure, certain embodiments may be disclosed in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the disclosed ranges. Accordingly, the description of a range should be considered to have specifically disclosed all the possible sub-ranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed sub-ranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Certain embodiments may also be described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the disclosure. This includes the generic description of the embodiments with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

Detailed Description

While oxidant-dependent indicators have been used to detect oxygen, the control of response times of the redox reactions in such indicators have not been studied in detail. Control and expansion of the range of reaction response times have also not

been studied in detail. Therefore, prior art oxidant-dependent indicators have not been useful as time indicators.

Exemplary, non-limiting embodiments of a time indicator will now be disclosed.

In an embodiment, there is provided an irreversible, colorimetric, oxidant-dependent time indicator comprising: a) a porous material substrate; b) one or more redox-active dyes; c) one or more reductants; d) one or more non-alkaline additives; characterized in that the components b) to c) are deposited on the substrate a) substantially in the absence of a polymeric material or thickener in the component matrix and substantially in the absence of an oxidant semipermeable coating layer on the substrate.

Advantageously, the disclosed indicator determines the progress of time by incorporating a mixture of redox-sensitive chemicals that may change color irreversibly as a function of the exposed time, and the level of oxidants present in the ambient atmosphere. Advantageously, the disclosed indicator may be relatively easy to manufacture and may be used in any ambient atmosphere, such as in modified atmosphere packaging.

Advantageously, the disclosed indicator possesses a variable shelf-life as required by its use. Advantageously, the shelf-life of the indicator may be controlled by varying the amounts of component b) and/or component c) and/or component d). Advantageously, control of the shelf-life of the indicator may not be dependent on additional polymeric material or thickeners to control the rate of diffusion of the oxidant to the redox-sensitive chemicals. Further advantageously, the components b), c) and/or d) may be substantially retained on the substrate a) with little or no need for thickeners.

The change in color results from the oxidation of the one or more redox-active dyes b). Component b) may be provided on the porous material substrate a) in the reduced form. Component b) in the oxidized form and component c) may be provided on substrate a) and, upon reaction of components b) and c), component b) may be present in the indicator in the reduced form prior to use. Component b) in the oxidized form and component c) may be provided on substrate a) and, upon reaction of components b) and c), component b) is present on the indicator in the reduced form prior to use. Component c) may maintain component b) in the reduced form on the substrate a) prior to use.

The reduced form of component b) may absorb a wavelength of light and thereby appear as a lighter or less obvious color or even colorless, while the oxidized form of component b) may absorb a wavelength of light and thereby appear as a darker or more obvious color. Alternatively, the reduced form of component b) may absorb a wavelength of light and thereby appear as a darker or more obvious color, while the oxidized form of component b) may absorb a wavelength of light and thereby appear as a lighter or less obvious color or even colorless. Examples of light

appearing as a darker color include blue light, green light, red light, violet light and the associated wavelengths exhibiting such colors.

For example, the reduced form of methylene blue, i.e. leuco-methylene blue, is colorless, while the oxidized form of methylene blue is blue in color.

The disclosed time indicator is provided as an irreversible indicator. This may require that reductants in the indicating environment are substantially absent when in use. As the disclosed indicator is effective for determining the duration of exposure of the indicator to the oxidant, thereby indicating the time elapsed, it is advantageous that the reaction of component b) with the oxidant is not reversible in the measuring environment. Component c) may reduce component b) to its reduced form and maintain component b) in the reduced form prior to use. When in use, component c) may first be oxidized upon exposure to the oxidant in the measuring environment. Component b) may be oxidized irreversibly only after all of component c) is oxidized.

The disclosed time indicator may be used with a wide range of oxidants or in an atmosphere comprising a wide range of oxidants. In examples, the oxidant is a fluid oxidant. In examples, the oxidant is a gaseous oxidant. Examples of oxidants include air, oxygen, oxygen-containing gas, peroxy compounds, hypochlorites, halogen oxides, halogen-containing gas, ozone and combinations thereof.

In an example, the oxidant is air or oxygen. Advantageously, the amount of oxygen in ambient air is relatively constant. Thus, the colorimetric change of the dye from one form to another may be directly related to how long the indicator is exposed to oxygen. An estimate of time elapsed from the point the indicator is exposed to air may be determined. Adjusting the molar ratio and the concentration of reducing agent relative to the redox active dye(s) may enable the indicator to control and expand the temporal range of the color change of redox-active dye.

The indicator or just the detection zone may be imaged at regular time intervals and analysed. The imaging may include scanning or taking a photograph under a controlled light environment and at a controlled distance. In an example, the analysis may be conducted with a software that will analyse the substrate in three color channels: Red, Green, and Blue, and quantify the intensity in each channel. The software used may be an image-analysis software such as ImageJ. In the case of methylene blue, the Red channel may be used because the color change of the indicator is most prominent in this channel. The correlation of intensity of the particular channel with time can be used for setting up a calibration graph. For example, the intensity of each pixel in the selected area is quantified on a greyscale that ranges from 0 to 255 and an average value of the intensity over the selected area is computed by the software. A threshold within the greyscale range may be defined, such as a maximal value of 220, which indicates the threshold of colorimetric change that is visible to the unaided eye. That is, at a maximal

threshold value of 220, the detection zone has no perceivable colorimetric change if the average value of the pixel intensity in the selected area is between the threshold value of 220 and the upper limit of 255. As methylene blue is oxidized, the average value of the pixel intensity in the red channel decreases as the color of the detection zone changes from white to blue. The change in color visible to the unaided eye may be quantified when the average value of the pixel intensity decreases below the maximal threshold value or increases above the minimal threshold value (if defined). When in use, the disclosed indicator may be compared against the calibration graph to determine the time elapsed.

In examples, the atmosphere in which the indicator is placed is at a relatively constant temperature. The ambient temperature may be room temperature, i.e. around 20°C to about 30°C. Depending on the application of the disclosed indicator, e.g. the atmosphere it is placed in, the amounts of the components of the indicator may be adjusted accordingly. For example, high humidity accelerates the rate of oxidation. Hence, in atmospheres of high humidity, the amount of component c) may be increased to stabilize the faster oxidation reaction rate of the dye. Furthermore the indicator may comprise further additives. Additives can be fillers, pH buffers and/or moisture control agents. The indicator may consist of components a) to d) only or additionally include the additives.

The porous material substrate a) may be any suitable substrate that can retain, contain, support or absorb components b), c) and/or d). In an example, the substrate a) may be paper or paper-based. In another example, the substrate a) may be a cellulosic material. In yet other examples, the substrate a) may be a synthetic polymeric membrane, fabric, such as woven fabric, mesh or a ribbon. In an example, the substrate a) is filter paper, such as Whatman® Grade 1 filter paper.

The redox-active dye b) may be water-soluble. The redox-active dye b) may be present in the indicator in an aqueous solution. The dye b) may be a cationic dye. The dye b) may be tetrazolium-based dyes, xanthenes, azines, oxazines, thiazines and combinations thereof. The dye b) may be a protein.

Examples of the redox-active dyes b) include Methylene Blue (C.I. Basic Blue 9), New Methylene Blue (C.I. Basic Blue 24), C.I. Basic Blue 3, phenosafranin, Capri Blue, Lauth's Violet, Methylene Green (C.I. Basic Green 5), Neutral Red, Safranin T (C.I. Basic Red 2), Indigo Carmine, Setoglaurine, Reactive Blue 19, Xylene Cyanol, Riboflavin, Erythrosine, Xylene Blue, Fast Green, Vad Green 1, Acid Blue 59 and Acid Red 51.

In an example, the at least one redox-active dye is methylene blue. The dye may be initially added to the indicator in its oxidized or reduced form.

The disclosed indicator may include other types of the dyes as long as the dye can be reduced and oxidized.

The concentration of dye b) may be present in the indicator at a concentration of about 0.05 grams/litre (g/L) or more.

The dye b) may be present in the indicator in a suitable amount depending on the size of the detection zone. For example, where the detection zone has an area of about 25 to 30 mm², the dye b) may be present in the indicator at an amount of about 5 µL to about 10 µL.

It may be appreciated that the amount and/or concentration of the components in the indicator may be adjusted in relation to each other, e.g. where the concentration of dye b) is increased, the reductant c) and additive d) may be increased and the calibration graph adjusted accordingly. It may also be appreciated that in a detection zone with a larger area, the amount and/or concentration of the components may be increased accordingly.

The reductant c) may be an acidic reducing agent. The term “acidic” refers to a compound that produces hydrogen ions when dissolved in an aqueous solution to result in a solution having a pH of less than 7. Advantageously, since oxidation of the reduced dye is accelerated at near neutral or alkaline conditions, e.g. pH of 7 or more, the disclosed indicator may have a longer shelf-life due to the reduction of oxidation rates. Advantageously, in examples where the indicator is to be used for a longer period of time or requires a longer response time, the oxidation rate of the reduced dye may be slowed down by the use of an acidic reductant. Short response times of less than 30 minutes may be needed in situations where exposure of the content in a sealed package to external atmospheric oxygen due to leakage or puncture in the package could compromise the quality or shelf-life of the content. In such case, the disclosed indicator should be placed inside the packaging. Long response times of 30 minutes or more may be useful, e.g., for monitoring the transport time of goods. In this case, the disclosed indicator could be placed on an external wall or surface of the packaging. As used in the present disclosure, a short response time is one that is less than 30 minutes, while a long response time is one that is 30 minutes or more.

The reductant may be a strong reducing agent or a weak reducing agent. The reductant may be an organic acid. The reductant may be ascorbic acid, erythorbic acid, enantiomers of ascorbic acid and erythorbic acid, hydrophilic phosphine and combinations thereof. The reductant may be L-ascorbic acid or D-erythorbic acid. The hydrophilic phosphine may be tris(2-carboxyethyl)phosphine. The reductant may be tris(2-carboxyethyl)phosphine hydrochloride (TCEP-HCl).

The reductant c) may be present in the indicator at a molar ratio of the reductant c) to the redox-active dyes b) at about 1:1 to about 125:1, or about 1:1 to about 50:1, or about 1:1 to about 20:1, or about 5:1, or about 10:1, or about 15:1, or more than 125:1. Where a short response time is required, the molar ratio of the reductant c) to the redox-active dyes b) may be about 2:1. Where a longer response time is

required, the molar ratio of the reductant c) to the redox-active dyes b) may be about 3:1 or more.

Advantageously, the molar ratio or concentration of reductant c) relative to the redox-active dye b) enables a user to control and expand the oxidation rates of the reaction, thereby enabling the user to control and expand the shades of the redox-active dye. The oxidation rate may be reduced and the corresponding change in color may therefore be lengthened such that the user can observe the various degrees of shades of colors between the color of the reduced form and the color of the oxidized form of the dyes a) during the course of use.

The reductant may be present in the indicator at a concentration of about 0.0005 moles (M) to about 0.2M.

The reductant may be present in the indicator in a suitable amount depending on the size of the detection zone. For example, where the detection zone has an area of about 25 to 30 mm², the reductant may be present in the indicator in an amount of about 2.5 µL to about 5 µL.

In an example, the reductant c) is TCEP-HCl. The TCEP-HCl may be present in the indicator at a concentration of about 0.001M to about 0.01M. The ratio of TCEP to HCl may be kept constant, e.g. at about 1:1. The TCEP-HCl may be present in the indicator in a suitable amount depending on the size of the detection zone. For example, where the detection zone has an area of about 25 to 30 mm², the TCEP-HCl may be present in the indicator in an amount of about 10 µL.

In a specific example where a response time of about 1 day is required, the ratio of TCEP to HCl to methylene blue may be about 8:8:1, while the ratio of TCEP-HCl to methylene blue may be about 8.3:1.

The one or more non-alkaline additives d) may advantageously be non-reducing. Component d) may be included to control the shelf-life of the indicator. Component d) may be included to further lengthen or expand the shelf-life of the indicator. Accordingly, if a short response time is required, component d) may not be included. It is known that non-reducing acids may be used to accelerate the reduction of methylene blue. Advantageously, the non-reducing property of component d) may assist in reducing the rate of oxidation of the reduced dye, yet may not affect the rate of the redox reaction occurring between the oxidant and the reduced dye. Component d) may be included to decrease the pH of the time indicator to decelerate the oxidation of the reduced dye b).

Component d) may be an acidic additive. Component d) may be a strong acid or a weak acid. Component d) may be selected from water-soluble strong acids. Examples of component d) include hydrochloric acid, hydrobromic acid, sulphuric acid, nitric acid, phosphoric acid, methanesulfonic acid, trifluoromethanesulfonic acid, hexafluorophosphoric acid, and tetrafluoroboric acid.

An increase in the relative amount of additive d) will lengthen the response time. For example, where the ratio of reductant c) to dye b) is kept constant, increasing the ratio of additive d) to reductant c) will lengthen the response time. In another example, where the ratio of additive d) to dye b) is kept constant, decreasing the ratio of additive d) to reductant c) will lengthen the response time.

The molar ratio of the acidic additives d) to the reductant c) may be about 5,000:1, or about 1,000:1, or about 500:1, or about 100:1, or about 50:1. In an example, where a short response time is required, the molar ratio of the additive d) to reductant c) may be about 4,000:1 when the ratio of reductant c) to dye b) is kept constant, whereas where a longer response time is required, the molar ratio of the additive d) to reductant c) may be about 2,000:1 at the same c:b ratio.

The molar ratio of the acidic additives d) to the redox-active dyes b) may be about 0:1 to about 10,000:1. Where a short response time is required, additive d) may not be included and thus, the molar ratio of the additive d) to dye b) may be about 0:1. In such instances where no additive d) is included, the rate of oxidation will depend solely on the molar ratio of reductant c) to dye b). Where a longer response time is required, the molar ratio of the additive d) to dye b) may be about 3000:1 or any larger ratio, for example about 3500:1 or more.

Advantageously, the molar ratio or concentration of the additive d) relative to the redox-active dye b) enables a user to control and expand the oxidation rates of the reaction, thereby enabling the user to control and expand the shades of the redox-active dye. Advantageously, the molar ratio or concentration of the additive d) relative to the reductant c) enables a user to control and expand the oxidation rates of the reaction, thereby enabling the user to control and expand the shades of the redox-active dye. Hence, an increase in ratio of d:b and/or an increase in ratio of d:c may lengthen the response time of the indicator as required as an increase in additive d) slows down the oxidation of the reduced form of dye b).

In an example, the non-alkaline additive d) is hydrochloric acid. The non-alkaline additive d) may be present in the indicator at a concentration of about 0.02M to about 4M. The hydrochloric acid may be present in the indicator in a suitable amount depending on the size of the detection zone. For example, where the detection zone has an area of about 25 to 30 mm², the hydrochloric acid may be present in the indicator in an amount of about 2.5 μ L to about 5 μ L.

In examples, the duration and rate of the colorimetric change are modulated by varying the formulation of reductant c) and additive d), as well as the amounts and/or concentrations of reductant c) and/or additive d) relative to the redox-active dye b) of the indicator.

The indicator may comprise a hydrophobic coating on the substrate a). Substrate a) may comprise a hydrophobic or a water-repellent coating. The hydrophobic coating may be in a hydrophobic zone on the substrate a) that is distinct from a detection

zone where components b), c) and/or d) are deposited. The hydrophobic coating may cover substantially the entire surface of the indicator or substrate a), leaving only the detection zone where components b), c) and/or d) are deposited exposed to the atmosphere. Advantageously, where the indicator is used in an atmosphere of high humidity, a reduced amount of moisture from the atmosphere may be absorbed by the substrate. Advantageously, changes in atmospheric conditions, such as changes in humidity, may be mitigated, resulting in lower variations of the redox reaction rate due to variations in atmospheric conditions and therefore, providing a more accurate time indicator.

Components b) and c) may be deposited in the detection zone of the hydrophobic-coated substrate a). The detection zone may be a hydrophilic detection zone. The detection zone may be a portion of substrate a) itself, i.e. not coated with a hydrophobic coating. The detection zone may be treated to enhance hydrophilicity. An exemplary procedure to increase hydrophilicity of the detection zone of the substrate is to expose the detection zone to oxygen plasma. The oxygen plasma treatment increases the number of hydrophilic functional groups on the surface of the substrate and enhances the hydrophilicity on the surface of the substrate. Such treatment should be performed before deposition of the components on the indicator.

Substrate a) may comprise one or more than one detection zones. In examples where there are multiple detection zones, each detection zone may comprise the same or different amounts of components b), c) and/or d) as other detection zones. In such examples, an array of detection zones is provided. The multiple detection zones may be arranged on substrate a) to provide a sequential color change, indicating the progression of time. For example, a first detection zone comprising components b), c) and/or d) providing a first oxidation rate is arranged before a second detection zone comprising components b), c) and/or d) providing a second oxidation rate slower than the first oxidation rate, which is in turn followed by a third detection zone comprising components b), c) and/or d) providing a third oxidation rate slower than the second oxidation rate. In this example, at any point in time, the first detection zone exhibits a shade of dye in the most advanced stage of oxidation as compared to that of the second detection zone, while the third detection zone exhibits a shade of dye that is least oxidized, i.e. most reduced. Accordingly, a sequential arrangement of detection zones may provide a visual indication of the progress of time. Measurement of time may be completed when all detection zones attain the same shade of oxidized dye, thereby signifying that the indicator has reached the end of its life and the maximum amount of time it can indicate.

The detection zone may be of a suitable size in relation to the substrate. In an example, the detection zone (circular) may be about 6 mm in diameter and the substrate (square) may be about 13mm × 13mm.

In examples where only one detection zone is provided, the shade of dye may be compared with a table of shades prepared beforehand to identify the time elapsed.

Advantageously, the disclosed indicator may provide a visually observable indication of the progress of time. Advantageously, a user may easily observe the progress of time.

The hydrophobic coating may be oxygen-permeable. The hydrophobic coating may comprise wax.

The hydrophobic coating may be provided on substrate a) using any method suitable for coating porous substrates. The hydrophobic coating may be printed on substrate a). The hydrophobic coating may be patterned on substrate a) to define the hydrophobic zone. The hydrophobic coating may be patterned on substrate a) to define the shape, size and/or dimension of the detection zone. The hydrophobic coating may be provided on substrate a) at an appropriate thickness to maintain oxygen permeability.

In an example, components b) and c) may be deposited in a hydrophilic detection zone of a wax printed substrate.

In examples, the components b) to d) are deposited on the substrate a).

The disclosed indicator may further comprise an alkaline additive or reagent deposited thereon in examples where the rate of oxidation of the reduced dye is to be accelerated or in examples where a short response time is required. The alkaline additive raises the pH of the indicator and may thereby accelerate the oxidation of both the reductant and the reduced dyes upon exposure to the oxidant. The alkaline additive may be non-reducing. Advantageously, the non-reducing property of the alkaline additive may assist in accelerating the rate of oxidation of the reduced dye, yet may not affect the rate of the redox reaction occurring between the oxidant and the reduced dye. The alkaline additive may be sodium bicarbonate or potassium bicarbonate. The amount of alkaline additive used may be adjusted in accordance with the required response time and may range from about 0 to about 1 M.

Components deposited on substrate a) may be dried for storage on substrate a). A hydrophilic liquid, such as water, may be added to the dried components at the detection zone when being activated for use, in order to dissolve and mix the components. The amount of liquid added is not particularly limiting, as long as the dried components can be dissolved.

In one embodiment, the disclosed time indicator may consist essentially of comprising: a) a porous material substrate; b) one or more redox-active dyes; c) one or more reductants; d) one or more non-alkaline additives; characterized in that the components b) to c) are deposited on the substrate a) substantially in the absence of a polymeric material or thickener in the component matrix and substantially in the absence of an oxidant semipermeable coating layer on the substrate.

In another embodiment, the disclosed time indicator may consist of comprising: a) a porous material substrate; b) one or more redox-active dyes; c) one or more reductants; d) one or more non-alkaline additives; characterized in that the components b) to c) are deposited on the substrate a) substantially in the absence of a polymeric material or thickener in the component matrix and substantially in the absence of an oxidant semipermeable coating layer on the substrate.

In an embodiment, there is provided an oxidant exposure time measuring device. The oxidant exposure time measuring device may comprise one or more of the disclosed indicators.

In examples, the device comprises an array of several indicators as disclosed herein. The indicators in the array may each comprise a single porous material substrate a), that is, a single layer or sheet of porous material substrate having components deposited thereon. More than one or several indicators as disclosed herein may be arranged in the form of a single layer of porous material as an array of indicators. The array of indicators may be sequentially arranged to provide a visual indication of the progress of time, as disclosed herein. The concentration ratios of redox-active dyes to reductants and additives may be varied among the plural indicators to obtain a colour change at different times of oxidant exposure.

In examples, the device is a two layer or multilayer device. The more than one or several indicators may be arranged on top of each other, covering each other, or co-axial with each other, thereby resulting in two or plural layers of porous material substrates or a sandwich of porous material substrates. The several indicators may be arranged on top of each other, covering each other, or co-axial with each other, such that the respective detection zones are partially or fully overlapping with each other. The device may comprise more than one sandwich of porous material substrates, wherein the plural sandwiches are arranged in an array.

Each layer may comprise the same or different formulations of components b), c) and/or d).

In examples, the plural layers of porous material substrate may further comprise one or more covering layers of porous material substrate comprising further additives. The further additives deposited on the covering layer may be dried for storage on the porous material substrate of the covering layer. In a particular example, one layer of indicator is covered with another layer of porous material that contains further additives. In one example, the further additive may comprise at least one alkaline additive. The alkaline additive may be one as disclosed herein. The amount of alkaline additive is dependent on the response time desired and may range from about 0 to about 1 M. Advantageously, the covering layer provides for examples where the rate of oxidation of the reduced dye is to be accelerated or in examples where a compressed or shortened response time is required.

In examples where there is more than one layer of indicator, the covering layer may be on top of, in between, or below the indicators. In examples where there is more than one covering layer, the plural covering layers may alternate between, or randomly inserted between, or placed on top or below the indicators.

The covering layer may be comprised of the same or different materials from the substrate a) of the indicator. The covering layer may comprise the same or different components deposited thereon as substrate a) of the indicator. The covering layer may comprise a hydrophobic coating and a hydrophilic zone where the further additives are deposited thereon, as described above. The indicator layer and the cover layer may include areas of hydrophilic zones or spots on the porous material which are separated by hydrophobic zones or areas created by wax printing. The layers may be arranged such that the respective hydrophilic zones are partially or fully overlapping with each other.

The layers may be bonded to each other to prevent displacement of the zones. The layers may be bonded to each other in a way that the hydrophilic areas where the redox-active dyes, reductants, non-alkaline and/or alkaline additives are deposited are in connection with each other. Any bonding may be suitable as long as the layers are held together. For example, the layers may be bonded to each other by tape, adhesive or lamination.

In an example, the indicator layer comprises the dried form of the reduced dye with an excess of reductant and non-alkaline additive, while the covering layer comprises the dried form of the alkaline additive. Water may be added to the dried components, thereby wetting the hydrophilic detection zones of the layers by capillary action to dissolve and mix the components by liquid diffusion across the layers.

Advantageously, the different formulations and different additives may be separated into different layers, thereby contributing to enhanced control of the properties of the device, such as the response time and the start point of the reaction.

Exemplary, non-limiting embodiments of a process for making the disclosed time indicator will now be disclosed.

In an embodiment, there is provided a process for making the disclosed time indicator. The process may include adding a solution of reductants and additives to a solution of a redox-active dye, and depositing the solutions on the porous material substrate. The process may alternatively include adding a solution of the reductants and additives to a pre-dried spot of a redox-active dye solution on the porous material substrate.

Depending on the application of the indicator, the solution of reductants and additives may be formulated accordingly, and as disclosed herein. For example, where the indicator is to be used to determine a time period of about 30 minutes or

less to several hours, e.g. 12 hours or less, to several days, e.g. about 5 days, or about 6 days, or about 10 days, the solution may comprise a higher amount of additive d) relative to the amount of reductant c). The solution may also require higher amounts of reductant c). In another example, where the indicator is to be used to determine a time period of less than about 30 minutes, less than about 20 minutes, less than about 10 minutes, or about 5 minutes or less, the solution may comprise an alkaline additive and a higher amount of reductant c) and additive d) relative to the amount of redox-active dye.

The process may comprise measuring the amount of oxidant in the environment where the indicator is to be used in to determine the amounts of reductants and additives to be added relative to the amount of dye.

The solutions of reductants, additives and/or redox-active dye may be aqueous-based solutions. The solutions may be deposited or loaded on the detection zone of the porous material substrate. The detection zone may be hydrophilic and therefore, may have affinity to the solutions. The solutions may be aqueous-based and therefore, may not have affinity to the hydrophobic areas of the substrate. The deposition spot of the redox-active dye on the porous material may be a hydrophilic zone separated by hydrophobic barriers from the rest of the porous material. Advantageously, the solutions may be added specifically at the detection zone.

The hydrophobic coating may be printed on the substrate. The pattern of hydrophobic areas may be introduced by wax printing on the substrate. The hydrophobic coating may be melted for a short period of time to pattern the shape, size and/or dimension of the detection zone.

The step of adding may be conducted by any method suitable to deposit solutions on a substrate. In an example, the solutions may be added by a pipette.

Upon contact of the solution of dye with the solution comprising the reductant, the dye may be converted into its reduced form. Alternatively, upon contact of the dried dye with the solution comprising the reductant, the dried dye may be dissolved and mixed with the reductant by diffusion to be converted into its reduced form.

The porous material substrate may comprise a reduced form of the dye deposited thereon.

A device as disclosed herein may be made by the disclosed process. The process for making the disclosed device may include depositing the solutions of reductants, additives and/or redox-active dye on the porous material substrate a) of the indicator and depositing a solution of an alkaline additive on a porous material substrate of another layer, e.g. the covering layer. The layers may be bonded to each other as disclosed herein.

The deposited solutions may be dried to aid in storage of the indicator. Advantageously, the dried deposited solutions may prevent further reactions from occurring, thereby retaining the dye in reduced form for later oxidation.

The process may be conducted in an ambient environment or normal atmosphere. The environment may or may not include oxygen. The component c) will ensure that component b) stays in reduced form. An environment comprising low, absent or controlled concentrations of oxygen may therefore not be required. Advantageously, active exclusion or removal of oxygen is not required. Advantageously, the disclosed process may not require specialized atmospheric conditions. The process may advantageously be conducted at room temperature. The process may be run at about 20 to 30°C, without the exclusion of oxygen.

The prepared indicator may be stored in a non-oxidizing environment, thereby retaining the dye in reduced form for later oxidation. The non-oxidizing environment may be an oxygen-impermeable enclosure, such as a container, packaging, satchel or pouch. The prepared indicator may be sealed in the non-oxidizing environment prior to use.

In an embodiment, a sealed oxidant-impermeable pouch comprising the disclosed indicator suitable for use in an irreversible, colorimetric oxidant-dependent reaction is provided.

In another embodiment, a kit of parts comprising the disclosed device together with an oxidant-impermeable container wherein the device is stored is provided.

Advantageously, the disclosed process may be economical as low material and fabrication cost may be incurred, as compared to prior art processes of making indicators based on diffusion control of oxygen through coatings or based on the embedding of redox-active dyes with or in a matrix of oxygen-semipermeable polymers.

Exemplary, non-limiting embodiments of a method of using the disclosed time indicator will now be disclosed.

In an embodiment, there is provided a method of using an indicator as disclosed herein to measure the oxidant exposure time. The method may include activating the indicator by exposing the indicator to a constant oxidant concentration in its environment. The method may include observing the irreversible, time-dependent change in colour after activation.

In examples, the method may comprise exposing the indicator to an environment comprising an oxidant. The environment may or may not have a constant oxidant concentration. The environment may comprise a constant or variable humidity levels.

In an example, the indicator may be exposed to an environment comprising a constant temperature, humidity and oxidant level.

The method may further comprise, prior to the activating step, a step of removing the indicator from a non-oxidizing environment or an oxygen-impermeable container. The removing step may comprise opening the oxygen-impermeable packaging.

The disclosed indicator, device and kit of parts may be used in a broad range of applications. For example, the disclosed products can be used at manufacturing/assembly lines to monitor the waiting time at each stage; in hospitals to record patient waiting time; in biological labs to record the incubation time for cell culture processes; and in packages of various goods (e.g. food, dry goods, biomaterials, flowers etc) to indicate the storage time, to name a few. The disclosed products may be pasted outside or on containers without contact with the goods.

It will be apparent that various other modifications and adaptations of the invention will be apparent to the person skilled in the art after reading the foregoing disclosure without departing from the spirit and scope of the invention and it is intended that all such modifications and adaptations come within the scope of the appended claims.

Brief Description of Drawings

The accompanying drawings illustrate a disclosed embodiment and serves to explain the principles of the disclosed embodiment. It is to be understood, however, that the drawings are designed for purposes of illustration only, and not as a definition of the limits of the invention.

Fig.1

[Fig. 1] is a calibration graph showing the response time (rate of oxidation) in days against the amount of reductant used in Example 2.

Fig.2

[Fig. 2a] is a graph showing six devices referred to in Example 2 having a varying amount of reductant of from 0 to 50 nmol at an amount of methylene blue of 1.6 nmol. The color changes of the six devices were monitored at six time intervals. [Fig. 2b] is a graph showing six devices referred to in Example 2 having a varying amount of reductant of from 12.5 to 375 nmol at an amount of methylene blue of 3 nmol. The color changes of the six devices were monitored at day 0 and day 7.

Fig.3

[Fig. 3a] is an illustration of an indicator according to an embodiment of the present disclosure that is exposed to ambient atmosphere. [Fig. 3b] is an illustration of a

two layer device according to an embodiment of the present disclosure that is exposed to ambient atmosphere. Like numerals denote like parts.

Detailed Description of Drawings

Referring to Fig. 3a, indicator 100 according to an embodiment of the present disclosure comprises a paper substrate 102. Substrate 102 comprises a hydrophobic zone of wax 104 and a hydrophilic zone 106 where reduced methylene blue dye and other components are deposited.

In use, the methylene blue dye is initially in its reduced form which is colorless. When exposed to oxygen in the ambient atmosphere for a typical time range of about 30 minutes to 6 days, the methylene blue dye is oxidized into its oxidized form which is blue.

Referring to Fig. 3b, a two layer device 200 according to an embodiment of the present disclosure comprises a paper substrate 202 and another paper substrate 212. Substrate 202 comprises a hydrophobic zone of wax 204 and a hydrophilic zone 206 where a solution of reduced methylene blue dye and other components are deposited. Substrate 212 comprises a hydrophobic zone of wax 214 and a hydrophilic zone 216 where a solution of non-reducing alkaline is deposited. The deposited solutions are dried and substrates 202 and 212 are bonded together by tape 220. The hydrophilic zones 206 and 216 of substrates 202 and 212 are aligned.

In use, water droplet 300 is dropped onto the hydrophilic zones 206 and 216 to dissolve the dried solutions and mix the non-reducing alkaline with the reduced methylene blue dye and other components. Upon exposure to oxygen in the ambient atmosphere, the methylene blue dye initially in its colorless reduced form is oxidized into its blue oxidized form. Due to the presence of the non-reducing alkaline, device 200 has a typical shelf-life of less than about 30 minutes.

Examples

Non-limiting examples of the invention will be further described in greater detail by reference to specific Examples, which should not be construed as in any way limiting the scope of the invention.

Example 1

In this example, a hydrophobic-coated substrate in accordance with an embodiment of the present disclosure was prepared.

Whatman® Grade 1 filter paper was chosen as the substrate of the indicator. A hydrophobic barrier made of wax was patterned onto the cellulosic substrate using wax printing. The patterned wax was subsequently melted at an elevated temperature of about 150-160°C for a short period of time of about 1-2 min to define the shape and dimension of the boundary of the detection zone(s).

Example 2

In this example, the relationship between the amounts of reductant comprised in the indicator against the oxidation response time was investigated.

The reductant used was ascorbic acid, the redox-active dye used was methylene blue and the non-alkaline additive used was hydrochloric acid.

Six devices in accordance with embodiments of the present disclosure were prepared. Each device was prepared by arranging six indicators comprising a sequential amount of reductant varied between 0 to 0.05 μmol in a line. The amount of methylene blue deposited on a circular detection zone of each indicator was kept at 0.0016 μmol , while the amount of hydrochloric acid was fixed at 1 μmol .

The device was exposed to an ambient indoor atmosphere at a temperature between 20-25°C and relative humidity at 50-70% for more than 5.5 days.

The results are shown in Figs. 1 and 2a.

In Fig. 1, a linear relationship between the amount of reductant and response time in the first 4 days can be observed.

In Fig. 2a, each column corresponds to the same device. It can be seen that as time progresses, the detection zones with lesser amounts of reductant were converted from white to blue, i.e. oxidized faster. Accordingly, it is evidenced that a lower amount of reductant relative to dye provides a shorter response time.

The experiment was repeated with an amount of methylene blue of 3.0 nmol and a varying amount of reductant of from 12.5 to 375 nmol. The concentration of hydrochloric acid was maintained at 1 μmol . The results are shown in Fig. 2b. It can be seen that as time progresses, the detection zones with lesser amounts of reductant were converted from white to blue, i.e. oxidized faster. At day 0, the color of the device having 12.5 nmol of reductant was darker, i.e. more blue, as compared to the device having 375 nmol of reductant. At day 7, the color of the device having 375 nmol of reductant was darker, i.e. more blue, than the same at day 0.

Claims

1. An irreversible, colorimetric, oxygen-dependent time indicator comprising
 - a) a porous material substrate;
 - b) one or more redox-active dyes;
 - c) one or more reductants;
 - d) one or more non-alkaline additives;characterized in that the components b) to c) are deposited on the substrate a) substantially in the absence of a polymeric material or thickener in the component matrix and substantially in the absence of an oxygen semipermeable coating layer on the substrate at the time of deposition, wherein at least one reductant is selected from the group consisting of ascorbic acid, erythorbic acid, enantiomers of ascorbic acid and erythorbic acid, hydrophilic phosphine and combinations thereof,

wherein the at least one non-alkaline additive is an acidic additive selected from water-soluble strong acids.
2. The time indicator according to claim 1, wherein at least one redox-active dye is methylene blue.
3. The time indicator according to claim 1, wherein the porous material substrate is paper or a cellulosic material.
4. The time indicator according to claim 3, wherein components b) to c) are deposited in a hydrophilic detection zone of a wax printed substrate.

5. The time indicator according to claim 1, wherein the molar ratio of the reductant to the redox-active dyes is about 1:1 to 125:1 or

wherein the molar ratio of the acidic additives to the reductants is about 5,000:1 to 1:1.
6. A method of using an indicator according to claim 1 to measure the oxygen exposure time wherein the indicator is activated by exposure to a constant oxygen concentration in its environment and its irreversible time dependent change in colour is observed after activation.
7. The method according to claim 6, wherein several indicators according to claim 1 are used with varied concentration ratios of redox-active dyes to reductants and additives to obtain a colour change at different times of oxygen exposure.
8. The method according to claim 6 or 7 to survey time in an environment with constant oxygen content in the air.
9. A process for making an indicator according to claim 1, characterized in that a solution of the reductants and additives is added to a solution of a redox-active dye and deposited on the porous material substrate; or solution of the reductants and additives is added to a pre-dried spot of a redox-active dye solution on the porous material substrate.
10. The process according to claim 9, wherein the process is run at about 20 to 30 °C without the exclusion of oxygen.
11. The process according to claim 9 or 10 wherein the deposition spot of the redox-active dye on the porous material is an hydrophilic zone separated by hydrophobic barriers from the rest of the porous material or

wherein the hydrophobic barriers

are introduced by wax printing on the substrate.

12. An oxygen exposure time measuring device comprising one or more indicators according to claim 1.
13. A device according to claim 12, wherein several indicators of claim 1 are arranged in form of a single layer of porous material as an array of indicators wherein the concentration ratios of redox-active dyes to reductants and additives are varied among the indicators to obtain a colour change at different times of oxygen exposure.
14. A two or multilayer device according to claim 12, wherein an indicator on one layer of porous material according to claim 1 is covered with another layer of porous material that contains further additives.
15. A two or multilayer device according to claim 14, wherein the covering layer comprises at least one alkaline additive.
16. A two or multilayer device according to claim 15, wherein the indicator layer and the cover layer include areas of hydrophilic spots on the porous material separated by hydrophobic areas created by wax printing; and the layers are bonded to each other in a way that the hydrophilic areas where the redox-active dyes, reductants, non-alkaline and alkaline additives are deposited are in connection with each other.
17. A kit of parts comprising a device according any of claims 12 to 16 together with an oxygen impermeable container wherein the device is stored.