Oxygen indicators and intelligent inks for packaging food

Andrew Mills

The detection of oxygen using optical sensors is of increasing interest, especially in modified atmosphere food packaging (MAP), in which the package, usually containing food, is flushed with a gas, such as carbon dioxide or nitrogen. This *tutorial review* examines the ideal properties of an oxygen optical sensor for MAP and compares them with those developed to date, including the most recent advances. The basic technologies underpinning the different indicator types are described, examples given and their potential for application in MAP assessed. This *tutorial review* should be of interest to the MAP industry and researchers in optical sensors and oxygen sensing.

Introduction

The main cause of most food-spoilage is oxygen,[1,2] since its presence allows a myriad of aerobic food-spoiling microorganisms to grow and thrive. Oxygen also spoils many foods through enzyme-catalysed reactions, as in the browning of fruit and vegetables, destruction of ascorbic acid and the oxidation of a wide range of flavours. Many oxidative food-spoiling reactions, including lipid oxidation, occur non-enzymically. Food-spoiling microorganisms are active above the freezing point of food, and most prevalent in foods of high water activity. In addition, the rates of growth of many spoilage organisms increase exponentially with temperature up to the point where they can be thermally disrupted or destroyed. Thus, keeping food chilled in an ambient atmosphere that is low in oxygen features strongly in most current, popular methods of food packaging and storage.

A common example of the latter is modified atmosphere packaging,[1,2] MAP, a process in which the atmosphere within the food package is flushed with an inert gas, such as nitrogen

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or carbon dioxide, reducing the oxygen content to typically 0.5–2%. Unlike nitrogen, carbon dioxide is classified as an *active* gas in food packaging, since it affects most food-spoiling microbes, reducing significantly their growth, even if some oxygen is present.[2] Both carbon dioxide and nitrogen slow the rates of respiring foods, such as fruits and vegetables and retard oxygen-based spoilage. As a consequence MAP is very effective at extending the shelf-lives of many foods and is widely used in food packaging. Some typical shelf-lives of MAPed and non-MAPed foods are given in Table 1. Table 2 provides regional details of the number of MAPed packages sold in 2004 and the predicted sales for 2007. The market is significant and growing, particularly in Japan and Western Europe.

After flushing a food package with an inert gas the level of residual oxygen in a package headspace is often still significant ($\geqslant 2\%$), due to poor gas flushing and the ability of the food to trap air. The level of residual air can also increase with time due to poor sealing and the permeation of oxygen through the package material from the surrounding air.[2] In order to overcome these problems, and ensure a very low residual level

Table 1 Typical shelf-lives of some common foods with and without MAP

Food	Lifetime when air-packaged (days)	Lifetime when MAPed (days)
Beef	4	12
Pork	4	9
Poultry	6	18
Bread	7	21
Coffee	1	350

Table 2 Current and predicted MAP and oxygen scavenger usage[3–5]

Country/Region	MAPed packages[a]		Oxygen scavengers[a]	
	2004	2007	2004	2007
Japan	20250	23000	13100	14400
US	5530	7240	1850	4500
Western Europe	15164	21716	2100	5700
TOTAL	40944	51956	17050	24600

[a] Units: millions.

of oxygen over the lifetime of the food package, an oxygen scavenger can be added to the packaging, usually in the form of a sachet or label. Typically, such sachets are used to maintain the level of oxygen in a food package at 0.1% or less. Table 2 provides estimated figures for the number of oxygen scavenger sachets and labels sold in Japan, the US and Western Europe in 2004 and 2007.[3–5] These figures reveal that the use of oxygen scavengers is most prevalent in Japan, where there is a real need for such technology given the hot and humid climate in the summer months. This market also benefits from having a large consumer base willing to pay higher prices for preservative-free foods with increased shelf-lives. In contrast to Japan, in both the US and Western Europe the use of such scavengers is comparatively low but, with increasing consumer awareness, expected to grow more significantly over the next few years.

The active ingredient in most oxygen scavenger sachets or labels for the food industry is ferrous oxide, FeO.[2,5] The sachets or labels are clearly labelled 'Do Not Consume', although the amount of FeO used is typically <200 times that of a lethal dose for an adult. In order to avoid the possible ingestion of the contents of an oxygen scavenger, which can be mistaken as a condiment despite its 'Do Not Consume' warning, such sachets are often placed under the peel-back lids of the packages. Fig. 1 illustrates a typical oxygen scavenger sachet concealed in a food package from a UK supermarket.

Fig. 1 Typical oxygen scavenger sachet in a food package; in this case, ham sold by Marks & Spencer, UK.

A major problem with MAP and/or oxygen scavengers is the lack of a simple, inexpensive oxygen indicator to show that the package is intact and that oxygen ingress is not significant. In this role an indicator would be effectively a leak and tamper detector and able to provide the food packager with 100% quality assurance as the packages left the factory. In addition, such technology would greatly reassure the consumer about the integrity of the product and the freshness of the food inside. This level of quality assurance is not possible currently in MAPed and O_2-scavenged foods. Instead, in a typical MAP food package line, one in every 300–400 packages is tested routinely by a trained technician, using expensive analytical systems such as FT-IR and/or GC; if a package is found to be faulty then the whole 300–400 food packages before are assumed to be improperly packaged and scrapped or repackaged. This situation is hardly ideal and, with the increasing growth in MAP and oxygen scavengers, there is a clear and recognisable need for a cheap, reliable, simple oxygen indicator for all MAPed and O_2-scavenged packages.

An ideal oxygen indicator

Before looking at what has been achieved in recent years with regard to the development of an oxygen indicator for food packaging, it is useful to consider what properties an ideal indicator should have. Maybe most importantly, it should be very inexpensive (*i.e. ca.* $\leqslant$1p per cm^2) and not add significantly to the overall cost of the package. It should also not require an expensive piece of analytical instrumentation for its interrogation and an untrained person should be able to check it. An ideal oxygen indicator should comprise non-toxic, non-water soluble components that have food contact approval, since the indicator will be placed inside the food package. It should have a very long shelf-life under ambient conditions and only be activated as an oxygen indicator when the package has been sealed and is largely or wholly oxygen-free. An ideal oxygen indicator should be tuneable with respect to oxygen sensitivity, *i.e.* utilise a chemistry that is readily and easily modified so that the indicator can be made to respond to changes at the 0.1% level (for oxygen scavenged packages) or at the 0.5–2% level, for non-scavenger MAPed packages.

An ideal oxygen indicator for the food packaging industry should also exhibit an irreversible response towards oxygen. To illustrate why this latter feature is so desirable it is worthwhile considering the response of a reversible oxygen indicator in a MAPed food package that, in a not too unlikely scenario, sometime later develops a small leak. Obviously, the indicator will show no oxygen is present in the package until the leak develops, at which time it will indicate the presence of oxygen. However, if the leak is small, it is very possible that the subsequent rapid increase in microbial growth will be such that within a short time the oxygen in the atmosphere in the package will be converted to carbon dioxide and the rate of bacterial metabolism will be matched by the rate of oxygen ingress. At this point the indicator will show, correctly, that little or no oxygen is present, even though the integrity of the package has been compromised and the food is now most probably not safe to eat. The above, not unlikely, scenario, of having a reversible indicator showing no oxygen on a

Table 3 Ideal and real oxygen indicator properties

| | Type of indicator | | | | |
| | Luminescence-based indicators | | Colorimetric-based indicators | | |
Ideal property	Intensity	Lifetime	O_2-binding complexes	Dark redox reactions	Light-driven redox reactions
Inexpensive	✗	✗	✓	✗	✓
Clearly discernible[a]	✗	✗	✓	✓	✓
Non-toxic	✓	✓	✗	✗	✓
Long ambient shelf-life	✓	✓	✗	✗	✓
In-pack activation	✗	✗	✗	✗	✓
Irreversible	✗	✗	✗	✗	✓
Tuneable	✓	✓	✗	✓	✓
Ink-based examples	✓	✓	✗	✓	✓
Non-water soluble	✓	✓	✓	✓	✗
References	7–12	7–13	14–16	17–22	25–30

[a] *i.e.* No supporting analytical instrumentation required, capable of assessment using the human eye.

compromised food package, is unacceptable and avoided by using an irreversible oxygen indicator.[6]

Finally, an ideal oxygen indicator should be easily incorporated into the food package and so is best applied as an ink, which must be printable on paper and plastic. In the food industry such an ink falls under the umbrella heading of intelligent packaging, which, by definition,[1,2] is any technology able to monitor and/or give information about the history and/or quality of the packed food. Thus, oxygen indicator inks that when printed produce indicators that provide information, or intelligence, about the level of oxygen in a food package, are often referred to as intelligent inks.[6]

Actual oxygen indicators

The above list of ideal oxygen indicator properties is unlikely to be addressed fully by any real indicator. However, such a list helps focus on what is required and identify the short-comings of the oxygen indicators developed to date. These indicators usually fall into one of two categories, namely: lumophoric or colorimetric. In the former, the luminescence intensity, or lifetime, of the indicator is measured and usually found to decrease with increasing partial pressure of oxygen. In the latter, a colour change is observed, in most cases due to either: (a) an oxygen-binding reaction, (b) a redox reaction or (c) a light-activated redox reaction. In the following sections, reported examples of these different types of oxygen indicator and their characteristics are described and compared with those of an ideal oxygen indicator in Table 3.

Luminescence-based oxygen indicators

Most optical sensors for oxygen are luminescence-based.[7] In such systems the luminescence associated with an electronically excited lumophore, L*, is quenched irreversibly by molecular oxygen, *i.e.*

$$\text{L}^* + \text{O}_2 \xrightarrow{\ k_Q(\text{O}_2)\ } \text{L} + \text{O}_2^* \qquad (1)$$
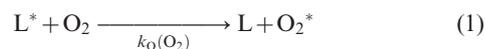
where k_Q (O_2) is the bimolecular rate constant for the quenching process.

The luminescent probe molecules are usually encapsulated in a gas permeable, ion-impermeable, material such as

silicone rubber, or an organic polymer, such as poly(vinyl chloride), to create thin film, oxygen indicators.[8–11] Although many different lumophores have been tested, one of the most popular is tris(4,7-diphenyl-1,10-phenanthroline) ruthenium(II) perchlorate, *i.e.* [Ru(dpp)$_3$](ClO$_4$)$_2$, where dpp is the complexing ligand, 4,7-diphenyl-1,10-phenanthroline. Table 4 provides some details of the photophysical properties of this complex.[11] This data shows that [Ru(dpp)$_3$](ClO$_4$)$_2$ is a highly luminescent complex ($\phi \cong 0.3$) with a long-lived excited state (*ca.* 5.3 μs), that is readily quenched by oxygen (k_Q (O_2) $\cong 2.5 \times 10^9$ dm^3 mol^{-1} s^{-1}).

In homogeneous solution the quenching of the luminescence of such a lumophore usually obeys the Stern–Volmer equation:

$$I_o/I = \tau_o/\tau = 1 + (K_{SV} \times p\text{O}_2) \qquad (2)$$

Where I_o and I, and τ_o and τ, are the luminescence intensities and excited state lifetimes of the lumophore in the absence and presence, respectively, of oxygen at a partial pressure, $p\text{O}_2$. K_{SV}, the Stern–Volmer constant[11] for the quenching reaction (1), depends directly upon τ_o, the rate constant for oxygen diffusion and the oxygen solubility constant for the reaction medium.[12] Fig. 2 illustrates a typical set of luminescence decay curves for a commercial oxygen indicator, the O$_2$xyDot™ (*vide infra*), comprising: [Ru(dpp)$_3$](ClO$_4$)$_2$ in a silicone rubber dot (5 mm diameter, 0.2 mm thick), as a function of increasing oxygen partial pressure.[13] From these results it can be seen that the lifetime of the luminescence, τ, *i.e.* the time taken for the luminescence to decay to 1/e of its initial value, decreases with

Table 4 [Ru(dpp)$_3$](ClO$_4$)$_2$: photophysical properties and example indicator sensitivities

Photophysical properties	
λ_{max} (absorbance) (nm)	452
λ_{max} (emission) (nm)	613,627
Luminescence lifetime, τ_o (μs)	5.3
Quantum yield of luminescence	~0.3
Oxygen quenching rate constant, k_Q (O_2) (10^9 dm^3 mol^{-1} s^{-1})	2.5
Optical sensor sensitivities [$p\text{O}_2$ ($S = \frac{1}{2}$) (Torr)] in different polymer media:	
Silicone rubber (RTV 118)	30
Poly(vinyl chloride)	132
Polystyrene	495

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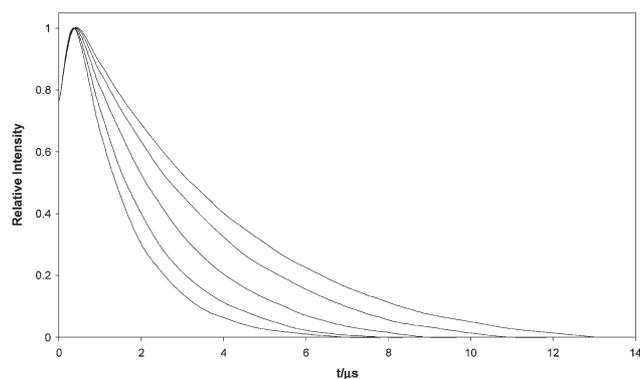
Fig. 2 Typical relative fluorescence intensity *versus* time profiles for an OxySense™ O₂xyDot™ exposed to different O₂–N₂ gas mixtures. The percentage O₂ in these mixtures was (from slowest to fastest decay): 0, 2.1, 6.3, 12.6 and 21.0%, respectively.[13]

increasing pO_2. Further work on this system shows that the quenching process, *i.e.* reaction (1), obeys the Stern–Volmer equation, *i.e.* eqn (2), and that, as a consequence, a plot of τ_o/τ *versus* pO_2 is a good straight line.[13]

Often, encapsulation of an oxygen-quenchable lumophore, such as $[Ru(dpp)_3](ClO_4)_2$, in a polymer matrix yields an oxygen indicator that does not obey the Stern–Volmer equation, but rather shows a negative deviation at high pO_2 levels. This effect is usually attributed to the micro-heterogeneous nature of the polymer, providing a range of different quenching environments. In such cases, one approach to providing a rough guide to the sensitivity of the oxygen indicator is the value of pO_2 at which $I_o/I = \tau_o/\tau = \frac{1}{2}$, *i.e.* the value of pO_2 when the luminescence intensity or excited state lifetime is half that of its value determined in the absence of oxygen.[11] This measure is referred to as pO_2 ($S = \frac{1}{2}$) and typical values of this parameter for three $[Ru(dpp)_3{}^{2+}]$-based oxygen indicators are reported in Table 4.[11] The values of pO_2 ($S = \frac{1}{2}$) determined for $[Ru(dpp)_3](ClO_4)_2$ in silicone rubber, poly(vinyl chloride) and polystyrene increase primarily due to a decrease in the oxygen permeabilities of the encapsulating polymers. Thus, it is possible to tune the sensitivities of these oxygen sensors by using different complexes, with different lifetimes, and/or using different polymer encapsulating media, with different oxygen permeabilities.[12]

Although it is much simpler and cheaper to measure luminescence intensities, rather than lifetimes, the former are affected by dye bleaching and leaching, temporal variations in the intensity of the excitation light intensity, and variations in the responsivity of the luminescence detector. Fortunately, lifetime measurements are not prone to these problems and, as a result, many researchers prefer to use this technique to probe luminescent oxygen indicators. The only problem with making lifetime measurements is equipment cost, but this has decreased markedly in recent years with the advent of blue and UV LEDs, coupled with phase modulation techniques. Indeed, *the* major current commercial oxygen sensor system used in food packaging research, OxySense™, uses lifetime measurements[13] to interrogate the luminescent probes.

As noted earlier, in the OxySense™ system, $[Ru(dpp)_3](Cl)_2$, is encapsulated in 5 mm diameter, 0.2 mm thick silicone rubber dots, called O₂xyDots™. In order to sense oxygen within a package, an O₂xyDot™ is attached inside and illuminated with a pulsed blue light from an LED, some of which is absorbed by the $[Ru(dpp)_3](Cl)_2$ in the dot. The resulting red emitted light is detected by a photodetector and the lifetime of the $[Ru(dpp)_3](Cl)_2$ extracted from the data. The level of oxygen in the package is then determined using an expression similar to eqn (2), relating lumophore lifetime to pO_2. The O₂xyDot™ can be calibrated using a one or two-point calibration procedure and is able to detect down to *ca.* 15 µmol dm⁻³ O₂ in water and 0.03% O₂ in the gas phase.[13] At present the equipment and O₂xyDots™ are too expensive to be considered for incorporation in all MAPed packages, but OxySense™ are expected soon to launch an inexpensive, *ca.* 1p, thin label, *ca.* 6 mm square, oxygen indicator, as an alternative to the O₂xyDot™, for embedding into MAPed packages. Such a label should allow food packagers to achieve 100% quality assurance in MAP and, with the possible launch of a hand-held reader in 2005, will provide a route for tamper detection at the retail level.[13]

Luminescence-based oxygen indicators can be formulated as inks, are tuneable with regard to oxygen sensitivity and now, with the imminent launch of the OxySense™ label, appear relatively inexpensive to produce. They do not, however, give a response that is visibly discernible and so require the use of analytical instrumentation that is still not inexpensive to interrogate the labels, rather than the human eye. They are also reversible in response, which is a feature noted earlier that is not considered particularly desirable in an oxygen indicator for food packaging.[6] However, despite these drawbacks, they do represent one of the few commercially viable approaches to oxygen detection and measurements in food packaging. The pros and cons of this type of oxygen indicator are highlighted in Table 3.

Colorimetric indicators based on oxygen-binding complexes

It is well known that a differently coloured product, oxyhaemoglobin, is formed when deoxyhaemoglobin combines with oxygen. The colour change is due to a shift in the porphyrin's Soret absorption band, from *ca.* 435 nm to 405 nm, as deoxyhaemoglobin is converted to oxyhaemoglobin. This established reaction forms the basis of one of the first reported colorimetric oxygen indicators, consisting of a layer of oxyhaemoglobin, immobilised on a cation exchange resin, positioned at the end of a fibre optic bundle.[14] The indicator was found to be reversible, and could be used to measure pO_2 values from 20–100 Torr. However, it was also found not to be very stable, lasting only 2 days when stored at room temperature, and requiring storage under anaerobic conditions to prevent the oxyhaemoglobin oxidising further to methaemoglobin.

Almost a decade later, in 1995, other workers developed a more robust dissolved oxygen indicator based on myoglobin (Mb) encapsulated in a sol-gel glass matrix.[15] Once again the colour changes were not striking, with the absorption maxima

for deoxyMb and oxyMb located at 432 and 418 nm, respectively. The basic process can be summarised as follows:

$$deoxyMb\ (Fe^{2+}) + O_2 \rightleftharpoons oxyMb\ (FeO_2)^{2+} \qquad (3)$$
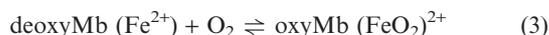
The indicator was prepared using metmyoglobin, metMb (Fe^{3+}), and required an initial reduction activation step to covert it to deoxyMb (Fe^{2+}), *i.e.*

$$metMb\ (Fe^{3+}) \xrightarrow[\text{sodium dithionite}]{Na_2S_2O_3} deoxyMb\ (Fe^{2+}) \qquad (4)$$
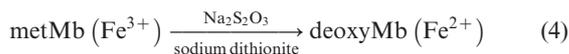
Given the oxygen sensitivity of this system, the activated, deoxyMb indicators required storing in sodium dithionite under argon at 4 °C. Although more stable than the earlier haemoglobin-based oxygen sensors, problems of storage and a less than marked colour change make this system an unlikely choice as an oxygen indicator.

Many synthetic oxygen-binding metal complexes have been studied, but only one appears to have led to the generation of a reproducible oxygen indicator. The successful metal complex, bis(histidinato) cobalt(II), $Co(His)_2$, is almost colourless in its unbound form, and pink (λ_{Max} = 408 nm) in its bound oxygen state, due to a charge transfer electronic transition between oxygen and the metal electronic levels.[15] The key oxygen-sensitive reaction can be summarised as follows:

$$2Co(His)_2 + O_2 \rightleftharpoons [Co(His)_2]_2O_2 \qquad (5)$$
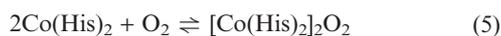
The indicator is reversible and appears to function best for dissolved oxygen measurements when immobilised on a thin-layer chromatographic (TLC) plate and covered with a thin film of silicone rubber, which acts as a gas-permeable, ion-impermeable membrane.[16] The reversible nature of this type of indicator, its poor colour change and sensitivity towards changes in pH and humidity, render it far from ideal as a potential oxygen indicator for MAP. The typical properties of such oxygen indicators based on an oxygen-binding complex are compared with those of an ideal oxygen indicator in Table 3.

Colorimetric redox dye-based indicators

The most commonly-employed leak indicator used in food packaging is a colorimetric redox dye-based indicator, the Ageless Eye™, produced by the Mitsubishi Gas Company to support its extensive range of Ageless™ oxygen scavengers,[17] an example of which is illustrated in Fig. 1. The Ageless Eye™ oxygen indicator, and other such colorimetric redox dye-based indicators, typically comprises a redox dye, such as methylene blue (MB), and a strong reducing agent, such as glucose in an alkaline medium.[6,17–19]

Typically, in the absence of a significant level of oxygen (typically $\leqslant$ 0.1%) a bleaching reaction,

$$D_{Red} + reductant \rightarrow D_{Red} + oxidised\ reductant \qquad (6)$$
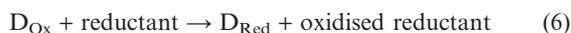
dominates and most of the dye lies in its reduced, often colourless, state, D_{Red}, rather than in its oxidised, more coloured form, D_{Ox}. In contrast, in the presence of a significant level of oxygen, (typically $\geqslant$ 0.5%), the oxygen-driven reaction,

$$D_{Red} + O_2 \rightarrow D_{Ox} + H_2O \qquad (7)$$

dominates and most of the dye lies in its oxidised, highly coloured form, D_{Ox}.

A general reaction scheme for this system is illustrated in Fig. 3 and, in the case of the Ageless Eye™: D_{Ox} = methylene blue, MB, which is blue, D_{Red} = leuco-methylene blue, *i.e.* LMB, which is colourless, and the reductant and its oxidised form are glucose and gluconic acid, respectively. A non-redox sensitive dye, Acid Red 52, is usually added to provide a background pink colour to the indicator[18,19] and all these components, plus magnesium or calcium hydroxide to provide an alkaline environment, are mixed and pressed together to form a pellet or tablet which is then encapsulated in a clear, plastic, oxygen permeable, ion-impermeable sachet. The latter is used to avoid any direct contact with food issues, since most of the components of the Ageless Eye™ pellet itself are water soluble and so susceptible to leaching upon direct contact with foods with high moisture content. The Ageless Eye™ oxygen indicator changes from purple to pink in colour within 2–3 hours, when the ambient gas phase is changed from air to one in which the level of oxygen is $\leqslant$0.1%, and reverts back to its original purple colour within 5 minutes upon exposure to an ambient atmosphere containing $\geqslant$0.5% oxygen.

The Ageless Eye™ indicator, in its oxygen permeable sachet, is relatively expensive to produce (*i.e.* typically retail cost: *ca.* 60p each) and so cannot be used in every oxygen-scavenged food package. They also require storage under anaerobic conditions, as they go 'off' in air, *via* reactions (6) and (7). They also are humidity sensitive, working best under humid conditions, and reversible in response. As a consequence, the use of the Ageless Eye™, and other colorimetric redox dye-based oxygen indicators in the food packaging industry,[18–22] for the most part has been confined to package research or the testing of packaging equipment. Cost, reversibility, storage and ease-of-use issues appear major barriers to their general incorporation in oxygen-scavenged food packages. The fact that they also look like a sweet does not help either! A comparison of the properties of this type of oxygen
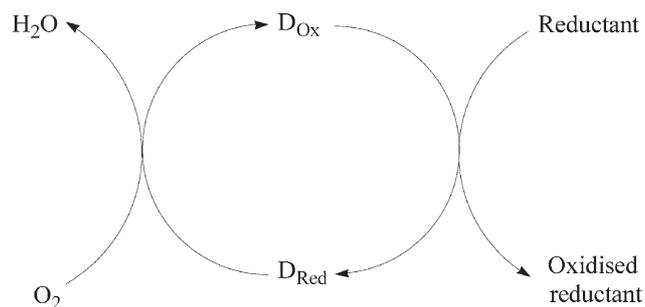
Fig. 3 Schematic illustration of the basic processes associated with a colorimetric redox dye-based oxygen indicator, where D_{Ox} and D_{Red} are the oxidised (usually highly coloured) and reduced (usually bleached) forms of the redox dye, respectively. The reductant is usually a reducing sugar in alkali, metal ion (often Fe^{2+}) or ascorbic acid.

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indicator with those of an ideal oxygen indicator is given in Table 3.

A rather nice example of the storage and use problems of such indicators is provided by the work of Eaton,[22] on an indicator film comprising: a redox dye, (2,6-dichloroindophenol) and a reducing sugar in alkali (fructose in tetrabutylammonium hydroxide), all encapsulated in a polymer (ethyl cellulose). Fig. 4 illustrates the typical variation in the absorbance of this indicator, measured at 640 nm, as a function of time upon its exposure to an alternating stream of air (1 min) and nitrogen (25 min) over a 20 h period. From these results, it is clear that after only 10 min exposure to air, *i.e.* 10 cycles, the rapid and clear response of this film to air begins to decrease significantly. The above feature of deteriorating responsivity, so typical of such sensors, arises from the combination of reactions (6) and (7). The latter cause a significant decrease in the reductant concentration and generation of interfering reaction products, upon prolonged exposure to a relatively high (21%) level of oxygen, both of which cause the indicator response characteristics to slow down significantly. As a consequence, such sensors require storage and initial deployment under anaerobic conditions, which can be costly[22] and makes them far from ideal as oxygen indicators.

Colorimetric light-activated, redox dye-based oxygen indicators

The reduction of many photoexcited dyes, S*, by a sacrificial electron donor, SED, like EDTA has been well studied[23–27] and can be summarised as follows:

$$S* + SED \rightarrow S^{\cdot-} + \text{oxidised SED} \qquad (8)$$

where $S^{\cdot-}$ is the semi-reduced form of the sensitiser, S.

In this reaction the SED is oxidised irreversibly. For example, EDTA is usually oxidised to glycolic acid and an amine residue. Under such conditions, $S^{\cdot-}$ usually disproportionates to form the leuco form of the sensitiser, S^{2-}, *i.e.*

$$2S^{\cdot-} \rightarrow S^{2-} + S \qquad (9)$$

Some of the most well studied dyes used in the above reaction include proflavine, riboflavin and uroporphyrin.[23–27] The photoreduction of such dyes, *via* reactions (8) and (9) is readily reversed by oxygen, *via* the following reaction:

$$O_2 + S^{2-} + 2H^+ \rightarrow H_2O_2 + S \qquad (10)$$
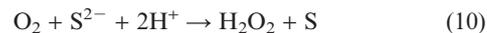
Thus, the electronically excited state of riboflavin is reductively quenched by EDTA to form leuco-riboflavin, *i.e.* 1,5 dihydroriboflavin, which then reacts readily with oxygen, if present, to regenerate the original dye, riboflavin.

Several colorimetric, light-activated, oxygen indicators for use with food packaging based on the above riboflavin/EDTA photosystem have been reported.[25,26] Typically the riboflavin and EDTA are dissolved in water, along with gelatin, which acts as the final encapsulation material and an antifoaming agent. This solution is then soaked into pieces of absorbent paper to produce a paper-based, orange-coloured, visible-light activated, oxygen sensitive film, which does not function unless and until it is irradiated with UV or visible light. Upon UV/Vis irradiation the film is bleached, provided no oxygen is present, and recovers its initial colour upon exposure to oxygen.[25,26]

Blinka *et al.*[25] were able to use the above technology to devise a method of detecting the flaws, such as pinholes and cracks, in an oxygen barrier polymer film, as well as a method for determining the overall permeability of the barrier.[25] The colour change associated with this system is not striking, but, fortunately, riboflavin is highly fluorescent in its oxidised state, S, and non-fluorescent in its reduced state, S^{2-}, rendering the above indicator system more effective when used in fluorimetric, rather than colorimetric, mode. These workers also noted that if a redox-indicator, such as methylene blue, was added to the formulation, then a good, UV/Vis activated colorimetric indicator, featuring the colour change blue (air) ↔ colourless (O$_2$-free), could also be created.[25]

The above technology has many attractive features, including: storability (it is not an oxygen indicator until activated with light and has no components that readily react with oxygen), a colorimetric optical change, irreversibility, reusability and potential production as an ink. However, it has a major drawback in that it is activated with visible as well as UV light, *i.e.* exposure to sunlight, or the bright lights associated with most supermarket food cabinets, would give rise to a bleached indicator, whether the environment it was in was oxygen free or not. The level of ambiguity this feature affords such indicators when used in food packaging is undesirable. Instead, what is required is an oxygen indicator ink that can be activated with only UV light, *i.e.* not visible light, since ambient levels of UV light, even in brightly-lit food cabinets, are generally very low.

In a recent communication we reported briefly on a novel intelligent ink for oxygen.[28] The ink can be used to produce a range of colorimetric, UV-activated, irreversible and reusable indicator films for oxygen that can be readily printed onto glass, plastic, paper and metal foil. The basic components of

Fig. 4 Typical absorbance (at 640 nm) *versus* time profiles for a dichloroindophenol/fructose/tetrabutyl ammonium hydroxide/ethyl cellulose oxygen indicator film exposed to a cycle of air (high absorbance) for 1 min and nitrogen (low absorbance) for 25 min over a period of 20 h. Note: within 10 min in air the response features of the oxygen indicator film begin to change markedly.[22]

this new type of generic oxygen indicator are as follows: a UV-only absorbing semiconductor photosensitiser, an appropriate redox indicator, a mild reducing agent, as the sacrificial electron donor and a polymer encapsulating material. All of the indicator components were chosen so that they were either soluble, or, in the case of semiconductor, readily dispersed in a common solvent, usually water, in order to create an oxygen intelligent ink that can be printed on a variety of typical substrates used in food packaging.[28]

Thus, a typical oxygen intelligent ink, based on the above general formulation, comprised: 5 g of a 5 wt% aqueous dispersion of anatase titania (TiO_2), 1 g of a 5 wt% aqueous solution of methylene blue (MB), 0.3 g of triethanolamine (TEOA) and 20 g of a 5 wt% aqueous solution of hydroxyethyl cellulose (HEC). The final solution, which needs to be well-mixed before use, but is otherwise very stable if kept in sealed bottles in the dark, is an example of an oxygen intelligent ink and will be referred to as such. The ink can be printed, or spun-coated, onto a variety of different substrates to create an UV-activated oxygen indicator. For simple laboratory demonstrations, a smeared drop of the above ink onto a glass slide, when allowed to dry generates a very effective oxygen indicator, the final appearance of which is as a blue, transparent film. The indicator is activated upon exposure to UV, but not visible, light. One inexpensive, artificial source of UVA light is the black fluorescent tube, referred to as a blacklight blue (BLB) tube, commonly found in hardware stores and used to highlight the presence of luminescent paints and dyes on clothes, banknotes and phosphorescent stickers and masks. Upon exposure of such an oxygen indicator to UV light, its colour quickly fades (e.g. 2.5 min with a 100 W BLB held ca. 20 cm away) and remains bleached in the absence of oxygen, but recovers under aerobic conditions when the source of UV is removed.

The above type of oxygen indicator does not change colour under either aerobic or anaerobic conditions. Indeed, it is not an oxygen indicator until it is activated with UV-light. Thus, unlike most colorimetric, redox dye-based indicators, such as the Ageless Eye™, this type of oxygen indicator has a very long shelf-life under dark, but otherwise ambient conditions, typically >1 year.

A typical set of absorbance versus time profiles for the UV-activated oxygen indicator are illustrated in Fig. 5. These profiles show that after UV-illumination, i.e. activation, the bleached film does not regain its original colour if the ambient gas phase is oxygen-free, curve (a), but does in air, curve (b) and oxygen, curve (c); with the latter showing the greatest rate of return. Other work shows that the indicator is not activated if any one component is omitted from the formulation given above.[28]

The UV-activated oxygen indicator works via a general mechanism summarised by the reaction scheme illustrated in Fig. 6. In brief, upon UV-excitation of the nanocrystalline, finely dispersed semiconductor (SC) powder particles encapsulated in the polymer film, electron–hole pairs are generated. The photogenerated holes are able to oxidise readily and irreversibly the SED present, leaving the photogenerated electrons to accumulate on the semiconductor particles [SC(e^-)]. These electrons reduce the highly-coloured
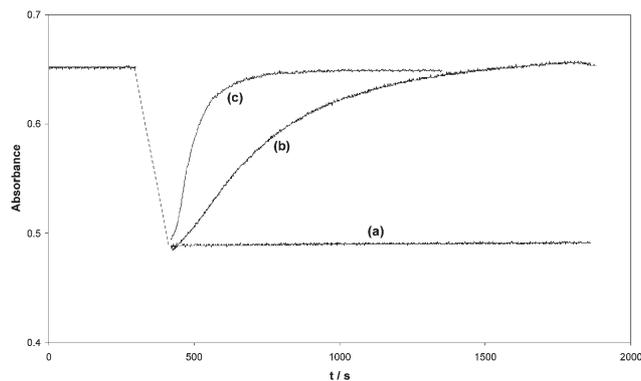
Fig. 5 Typical absorbance (at 610 nm) versus time profiles for a typical UV-activated oxygen indicator, recorded under the following different gaseous environments: (a) N_2, (b) air and (c) oxygen. Each film was initially left to stand for 3 minutes, before being exposed to a short burst (2.5 min) of UVA light (provided by a 100 W BLB), to activate, i.e. bleach, the film (broken line). The subsequent recovery of the original colour/absorbance of the indicator film then depended upon the percentage O_2 in the ambient gas phase.[28]

redox-indicator (D_{Ox}) to its usually bleached form (D_{Red}). This latter species is readily oxidised back to D_{Ox} by oxygen. With respect to the general scheme illustrated in Fig. 6, for the UV-activated oxygen indicator reported here, SC = TiO_2, D_{Ox} = MB, D_{Red} = LMB, SED = TEOA, with all these components encapsulated in a polymer = HEC.

This type of UV-activated oxygen indicator is irreversible, in that it requires UV-activation in order to make it function and once it has responded to the presence of oxygen and returned to its original colour, this state persists regardless of any subsequent changes in the oxygen level. It can only be made to function again as an oxygen indicator if it is reactivated upon exposure to another burst of UVA light.

Other work shows that, after UV-activation, the rate of recovery of the colour (i.e. absorbance) of the indicator film is
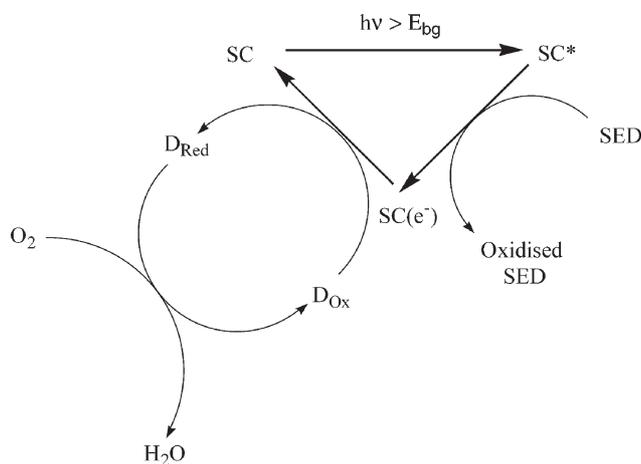
Fig. 6 Schematic illustration of the basic processes associated with a UV-activated oxygen indicator, used to generate the data in Fig. 5, which has the following generic formulation: semiconductor (SC), sacrificial electron donor (SED), redox indictor (initial, highly coloured oxidised form: D_{Ox}, photo reduced, usually bleached, reduced form: D_{Red}).

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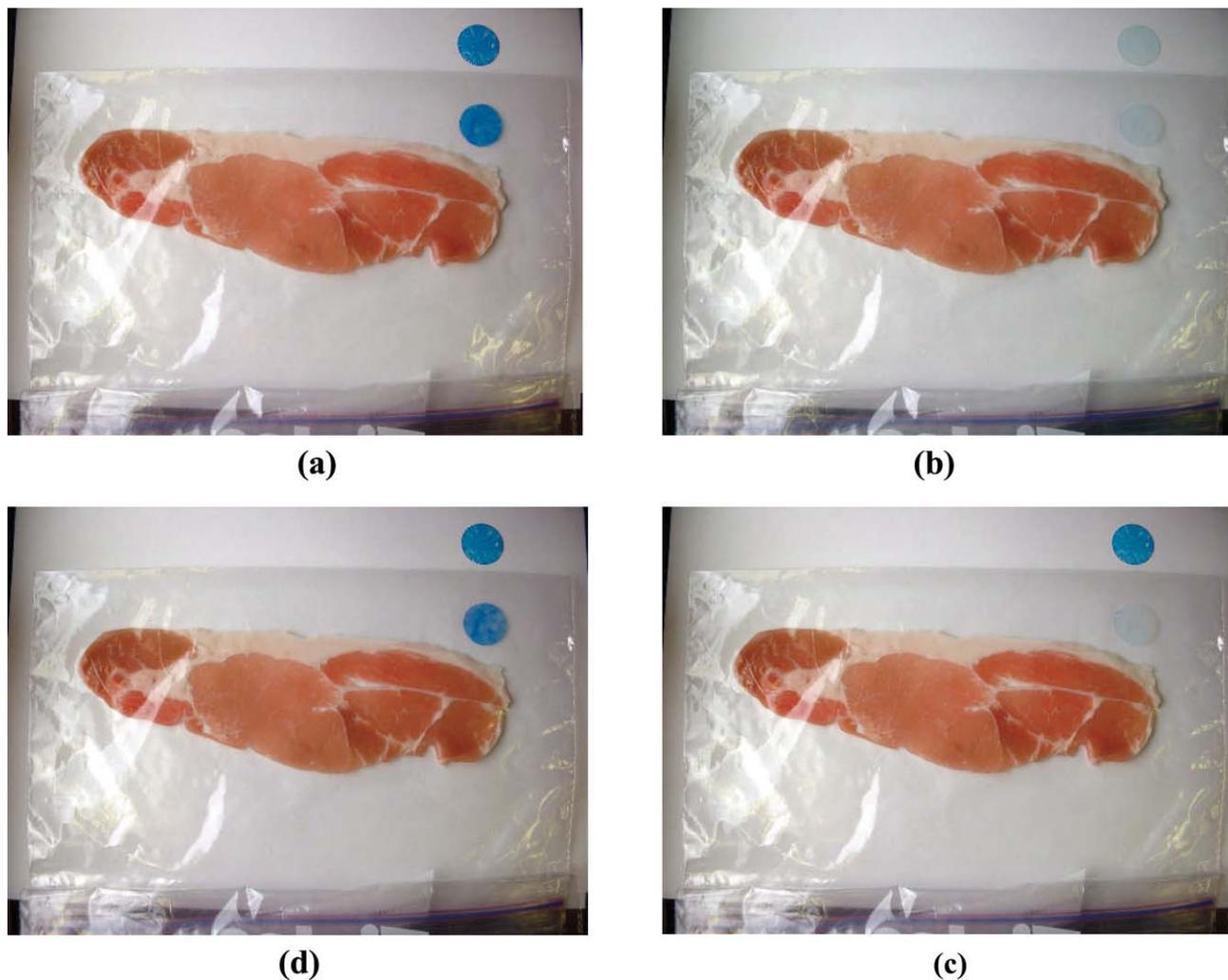
Fig. 7 Photographs of two typical blue-coloured UV-activated oxygen indicators with the formulation: MB/TiO$_2$/TEOA/HEC, one placed inside and the other outside a plastic package flushed with CO$_2$ and sealed. In (a) the package had just been sealed and the two indicators are blue. The two indicators were then bleached by a short (2.5 min) burst of UVA light (b). The indicator outside the package regained its original colour within minutes, whereas, 48 h later, the indicator inside remained bleached (c). Finally, upon opening the package, the indicator within regained its original colour in response to the ingress of oxygen (d).

proportional to the concentration of oxygen in the ambient gas phase. Thus, by monitoring this rate, a measure of the level of oxygen present can be gleaned. By using other redox dyes, with potentials more positive than that of MB, it is also possible to create less sensitive UV-activated oxygen indicators and *vice versa*. The sensitivity of the system can also be altered by using polymers of different oxygen permeability. The type of UV light required for activation can be varied by using semi-conductors with different band gaps. Thus, the range of oxygen indicators that can be created using the above technology is substantial.

Finally, an example of the possible use of this type of oxygen indicator in a food package is provided by the sequence of photographs in Fig. 7. In this experiment a piece of fresh, uncooked bacon was packaged under carbon dioxide and sealed with a typical intelligent oxygen indicator inside and one outside, picture (a). Upon irradiation of both indicators with UVA light, (2.5 min, 100 W BLB lamp) the indicators were bleached, picture (b). Within a few minutes the oxygen indicator outside the package regained its original colour, but even after a period of 48 h the indicator inside the package remained in a bleached state, indicating the absence of oxygen, picture (c). Finally, upon opening the package 48 h later, the indicator inside reverted rapidly (within 10 minutes) to its original colour, due to the ingress of air, picture (d). In other work the activated indicator has been sealed within an oxygen free package for several weeks without showing any change in colour until air was let in. At present, there appears to be no obvious long-term stability issues with this indicator in its activated or deactivated form.

Comparison of the properties of the different oxygen indicator technologies in Table 3, reveals that this latest UV-activated, colorimetric, irreversible, reusable generic type of oxygen indicator and ink has many attractive features. The advent of this technology has attracted a notable degree of interest from the food packaging industry.[28–30] However, at present only water-based inks have been reported, which renders the technology incompatible with respect to direct

food contact. Instead, any indicator would need to be covered with an oxygen-permeable, water-impermeable layer. Such a laminated structure is not unusual in food packaging. Promisingly, recent work by this group has demonstrated that a parallel set of organic solvent-based oxygen indicator inks can be generated.

Conclusion

Oxygen indicator inks are just one group of many intelligent inks, including: time–temperature, pH, off-gas and food-spoiling bacteria indicators, which are being developed at present. As the use of MAP and oxygen scavengers increases and consumers demand more information about the food they eat, especially with regard to its quality and security, so will the demand increase for the overt use of intelligent inks, such as the oxygen indicators described here.

Luminescence-based indicators, such as the OxySense™ system, are already being used in food-packaging research. However, their cost is currently prohibitive for widespread use and they require expensive lifetime measuring systems to make reliable measurements. Colorimetric sensors, based on simple reversible redox chemistry, such as the Ageless Eye™, are also well-established in food packaging research. However, these also are expensive and require storage and handling under anaerobic conditions; such features pose a major problem to their routine use in MAP. The underlying technology associated with the recent advent[28] of a range of UV-activated, colorimetric oxygen indicators appears to address many of the problems associated with commercial indicators, especially for use in the food-packaging industry. Such sensors appear to have great commercial potential and are likely to feature strongly in future smart packaging.

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