

A WATER-DISPERSIBLE OXYGEN-SENSITIVE FLUORESCENT COMPLEX
AND OXYGEN-SENSING FILMS FOR SMART PACKAGING

Cross-Reference to Related Application

5 [0001] This application claims the benefit of priority of Singapore Patent Application No. 10202008922T, filed 11 September 2020, the content of it being hereby incorporated by reference in its entirety for all purposes.

Technical Field

10 [0002] The present disclosure relates to an oxygen sensor and uses of the oxygen sensor. The present disclosure also relates to a method of forming the oxygen sensor.

Background

[0003] Monitoring dissolved oxygen (DO) level is considerably a significant task in
15 various fields, such as industrial water treatment, food and fermentation industry, aquaculture, environmental monitoring, clinical analysis, etc.

[0004] For example, vacuum and modified atmosphere (MAP) packaging is considerably a very fast-growing industrial trend that provides a noticeable extension of a product shelf life. Loss of the package tightness and oxygen penetration may cause
20 changes in the internal atmosphere initiating bacterial growth. As a result, the product shelf life may decrease, and spoilage may easily occur before the expiry date stated by a manufacturer. Some common reasons for oxygen penetration in a package may include defects in seals, mechanical damages, manufacturing defects in the packaging material (microcracks, uneven film thickness), and/or oxygen migration through the
25 packaging material.

[0005] In another example, the dissolved oxygen level in water may be measured continuously, or sampled, by use of oxygen sensor. Traditionally, three categories of DO sensor tend to be available, which may include galvanic sensor, polarographic sensor, and optical sensor. Both galvanic and polarographic sensors are often
30 electrochemical sensor, which may function when oxygen diffuses from sample to the sensor and undergoes reduction reaction on the sensor surface. Compared to the galvanic and polarographic sensor, optical DO sensor tends not to consume oxygen

during measurement. A significant part of the optical DO sensor is a sensor film (e.g. a membrane), which may contain an oxygen sensitive fluorescent dye. The interaction between oxygen and the fluorescent dye quenches the emission intensity of dye. The quenched emission intensity may be correlated to oxygen concentration, and the correlation may be linearized to allow the calculation of oxygen concentration in water. However, the DO sensors mentioned above may not be adequate for measuring oxygen in a headspace.

[0006] There is thus a need to provide for a solution that addresses one or more of the limitations mentioned above. The solution should at least provide for an optical sensor operable to measure dissolved oxygen as well as oxygen in a headspace. The solution may provide a non-destructive, cost-effective, and efficient technology to control the oxygen content inside packages throughout the entire supply chain and during the packages' lifecycle.

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Summary

[0007] In a first aspect, there is provided for an oxygen sensor that includes:

- a multilayered polyelectrolyte film;

- a dye carrier including a dye which (i) emits fluorescence in response to a stimuli and (ii) exhibits fluorescence emission inversely proportional to the amount of oxygen present;

- wherein the dye carrier is incorporated in the multilayered polyelectrolyte film,

- and

- wherein the dye carrier includes a particle covalently attached with the dye.

[0008] In another aspect, there is provided an oxygen sensor film tag including:

- the oxygen sensor described in various embodiments of the first aspect;

- a food-safe barrier film which is arranged to face food in a food package;

- a transparent protective film arranged distal to the food-safe barrier film; and

- an adhesive layer for adhering the film tag to a surface of a food packaging,

- wherein the oxygen sensor is arranged between the food-safe barrier film and the transparent protective film.

[0009] In another aspect, there is provided a method of forming the oxygen sensor described in various embodiments of the first aspect, the method includes:

providing a template having depressions arranged as an array;
depositing polyelectrolytes on the template to form multiple layers of polyelectrolyte;
contacting a dye carrier with the multiple layers of polyelectrolyte; and
5 sealing the multiple layers of polyelectrolyte to have an array of hollow chambers defined therein, wherein the dye carrier is confined in the hollow chamber.

Brief Description of the Drawings

[0010] The drawings are not necessarily to scale, emphasis instead generally being
10 placed upon illustrating the principles of the present disclosure. In the following description, various embodiments of the present disclosure are described with reference to the following drawings, in which:

[0011] FIG. 1A shows a scanning electron microscope (SEM) image of hollow silica. Scale bar denotes 10 μm .

15 [0012] FIG. 1B shows a SEM image of hollow silica with TCPP. Scale bar denotes 10 μm .

[0013] FIG. 2 shows how the polymers and mold are stacked for imprinting patterns on the PMMA template film, i.e. silicon (Si) wafer / polyethylene film (PE film) / poly(methylmethacrylate) (PMMA) / silanized mold (Si mold) / PE film.

20 [0014] FIG. 3 shows sedimentation of the ink complex of PcTCPP-His used in fabrication of a membrane film sensor of the present disclosure via screen printing.

[0015] FIG. 4A is a photograph showing four samples fabricated based on a screen printing approach of the present disclosure.

25 [0016] FIG. 4B is a photograph showing printed samples on a polyethylene (PE) substrate.

[0017] FIG. 5 is a photograph showing samples obtained via a molding method using a 96 wells plate.

[0018] FIG. 6 is a schematic illustration of a chamber of the present disclosure configured to carry out oxygen measurement using oxygen sensors of the present
30 disclosure.

[0019] FIG. 7A shows various fluorescent spectra obtained using sensor samples measured at different oxygen concentrations in a headspace.

[0020] FIG. 7B shows a calibration curve of the sensor.

[0021] FIG. 8 shows various fluorescent spectra obtained using sensor samples measured at different oxygen concentrations in a fluid.

[0022] FIG. 9 shows a handheld scanner used in oxygen sensing tag calibration.

5 [0023] FIG. 10 shows a fluorescent spectra processed using Python, wherein the measurement was obtained using sensors of the present disclosure. The area ratio between 700 nm peak (from 560 nm to 700 nm) denoted as S_{peak} and total area under spectrum denoted as S_{total} were used to calibrate the sensors. The area ratio was calculated as $R = (S_{\text{peak}}/S_{\text{total}})*1000$.

10 [0024] FIG. 11 shows a plot of ratios obtained using the spectra of FIG. 10 against oxygen concentration.

[0025] FIG. 12 demonstrates the present sensor and handheld scanner using vacuum packed rice as one non-limiting example.

15 [0026] FIG. 13 demonstrates the present sensor (left image) and handheld scanner (right image) using sealed packages of fresh meat.

[0027] FIG. 14 is a plot of oxygen migration through a packaging material of a vacuum packed rice.

[0028] FIG. 15 demonstrates the present sensor (left image) and handheld scanner (right image) using vacuum insulation panel.

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Detailed Description

[0029] The following detailed description refers to the accompanying drawings that show, by way of illustration, specific details and embodiments in which the present disclosure may be practised.

25 [0030] Features that are described in the context of an embodiment may correspondingly be applicable to the same or similar features in the other embodiments. Features that are described in the context of an embodiment may correspondingly be applicable to the other embodiments, even if not explicitly described in these other embodiments. Furthermore, additions and/or combinations and/or alternatives as
30 described for a feature in the context of an embodiment may correspondingly be applicable to the same or similar feature in the other embodiments.

[0031] The present disclosure relates to an oxygen sensor. The term “oxygen sensor”, “oxygen sensing element”, and “oxygen-sensitive membrane film sensor” are interchangeably used herein. For brevity, the oxygen sensor may be termed herein sensor.

5 [0032] Briefly and as non-limiting examples, the present sensor may be an optical oxygen-sensitive membrane film sensor (MFS). The MFS may include a micron-sized hollow or CaCO_3 templated silica (HSi) particles having an oxygen responsive fluorescent dye (e.g. tetrakis(4-carboxyphenyl)porphyrin platinum complex (PtTCPP)) covalently attached, which is either embedded within layers of a flat semi-permeable
10 multilayered polyelectrolyte film or loaded in a multilayered polyelectrolyte having chambers therein uniformly formed as an array. The fluorescent dye may be a hydrophobic dye. The hydrophobic dye may be attached to the micron-sized HSi particles to obtain a water-dispersible complex (PtTCPP-HSi) for embedment or encapsulation in the multilayered polyelectrolyte film or chambers. The multilayered
15 polyelectrolyte film and the multilayered polyelectrolyte may be a flat membrane or film impermeable with respect to the dye complex (e.g. PtTCPPHSi complex), i.e. traps the dye and avoid direct contact and potential contamination of the product or environment with the dye molecules. The chambers in the multilayered polyelectrolyte may also be impermeable with respect to the dye complex and hence trap the dye therein.
20 The emission intensity of the dye decreases with increasing oxygen concentration due to quenching of fluorescence of the PdTCPP-HSi complex by the oxygen.

[0033] The present oxygen sensor is usable as a sensing element incorporated in a film tag. The film tag may include a food contact approved functional barrier film, a transparent protective film, and an adhesive layer. For signal amplification, 3 layers of
25 the MFS may be included in a single film tag. The film tag can be further integrated to a vacuum or MAP packaging to measure oxygen content inside the package and monitor the package integrity through its life-cycle.

[0034] For detecting oxygen level via the oxygen sensor, a fluorescent spectroscopy device equipped with a reflecting probe can be used to excite the fluorescent dye and
30 measure the emission signal from oxygen sensor, wherein the emission signal is correlated to the oxygen content inside the package. As such, the present oxygen sensor is termed an “optical oxygen sensitive membrane film sensor”.

[0035] Details of various embodiments of the present oxygen sensor, its uses and methods of forming the oxygen sensor, and advantages associated with the various embodiments are described below and demonstrated in the examples section.

[0036] In the present disclosure, there is provided an oxygen sensor. The oxygen sensor
5 may include a multilayered polyelectrolyte film, a dye carrier that includes a dye which (i) emits fluorescence in response to a stimuli and (ii) exhibits fluorescence emission inversely proportional to the amount of oxygen present. The dye carrier may be incorporated in the multilayered polyelectrolyte film, and the dye carrier may include a particle covalently attached with the dye. The term “inversely proportional” herein
10 means that the intensity of the fluorescene emitted by the dye (i) decreases with increasing presence of oxygen and (ii) increases with decreasing presence of oxygen.

[0037] In various embodiments, the multilayered polyelectrolyte film may be or may include a semi-permeable film containing multiple layers of polyelectrolyte absent of hollow chambers, or a film containing multiple layers of polyelectrolyte having hollow
15 chambers therein arranged in an array. In various embodiments, the dye carrier may be confined within (i) the multiple layer of polyelectrolyte and/or (ii) the hollow chambers. In various embodiments of the semi-permeable film containing multiple layers of polyelectrolyte, the dye carrier may be embedded within the multiple layers of polyelectrolyte. The multiple layers of polyelectrolyte may be impermeable with
20 respect to the dye carrier.

[0038] In various embodiments, the multiple layers of polyelectrolyte may include poly(allylamine hydrochloride) and/or poly(sodium 4-styrene sulfonate).

[0039] The oxygen sensor may further include poly(ethyleneimine). The multiple layers of polyelectrolyte may be formed on the poly(ethyleneimine).

25 [0040] In various embodiments, the dye may include tetrakis(4-carboxyphenyl)porphyrin platinum complex. Other oxygen sensitive dye may be used. Said differently, dye carriers having other oxygen sensitive dye(s) may be used.

[0041] In various embodiments, the particle may include porous hollow silica particle. The porous hollow silica particle may be mesoporous hollow silica particle. The porous
30 hollow silica particle may have a raspberry-like structure. A particle having a raspberry-like structure refers to a particle that has an uneven porous surface or shell, where the uneven surface may arise due to random disposition of other smaller sized particles on

one core particle during formation of the raspberry-like particle, rendering the surface of the particle uneven. Such hollow particles may be termed microraspberries. In certain non-limiting embodiments, the particle may include mesoporous hollow silica particle. The term “mesoporous” herein refers to a material containing pores with diameters ranging from 2 nm to 50 nm.

5 [0042] The present disclosure also relates to an oxygen sensor film tag. Embodiments and advantages described for the present oxygen sensor of the first aspect can be analogously valid for the present oxygen sensor film tag subsequently described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples, they shall not be iterated for brevity.

10 [0043] The oxygen sensor film tag may include the oxygen sensor described in various embodiments mentioned above, a food-safe barrier film which may be arranged to face food in a food package, a transparent protective film arranged distal to the food-safe barrier film, and an adhesive layer for adhering the film tag to a surface of a food packaging. The oxygen sensor may be arranged between the food-safe barrier film and the transparent protective film. The food-safe barrier film protects the food from direct contact with the oxygen sensor. The transparent protective film may face the packaging material to protect the oxygen sensor film from external damage. The oxygen sensor may be sandwiched between and/or in contact with one or both the food-safe barrier film and the transparent protective film. The term “food-safe” herein means that the substance or material does not contaminate or damage the food in a way that compromises consumption of the food. A portable fluorescent spectroscopic detector (e.g. a handheld scanner) may be used to activate (i.e. provide a stimuli to have the oxygen sensor emit fluorescence) and detect the level of fluorescence emission. Such a detector may have a reflecting probe, which is operable to excite the dye and measure the fluorescence emission signal from the oxygen sensor. The fluorescence emission signal may then be correlated to the oxygen content detected, for example, inside the package. In various instances, the stimuli may be a light having a specific wavelength for exciting the fluorescent dye. Such a wavelength may be referred herein as an “excitation wavelength” that may be specific for identifying one or more particular molecules. In certain non-limiting instances, the portable fluorescent spectroscopic

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detector is operable to activate the present oxygen sensor by emitting light having an excitation wavelength of, for example, 520 nm.

[0044] The present disclosure further relates to a method of forming the oxygen sensor. The method may include providing a template having depressions arranged as an array, depositing polyelectrolytes on the template to form multiple layers of polyelectrolyte, contacting a dye carrier with the multiple layers of polyelectrolyte, and sealing the multiple layers of polyelectrolyte to have an array of hollow chambers defined therein. The dye carrier may be confined in the hollow chamber. Embodiments and advantages described for the present oxygen sensor of the first aspect can be analogously valid for the present method subsequently described herein, and vice versa. As the various embodiments and advantages have already been described above and in the examples, they shall not be iterated for brevity.

[0045] In the present method, the template may be a polymer template. In various embodiments, the template may be a sacrificial template, i.e. it is removed or not present in the present oxygen sensor. In various embodiments, the template may be poly(methylmethacrylate) (PMMA). The template may be formed by placing a polymer on a mold (e.g. silicon mold) having depressions arranged in an array and then applying pressure on the template to have the trenches imprinted on the template. This helps to form the array of chambers. In certain instances, a template without depression may be used if simply a multilayered polyelectrolyte film (without the chambers) is required.

[0046] After the template is formed, a base layer of poly(ethyleneimine) may be first deposited. In other words, the present method may further include forming a layer of poly(ethyleneimine) on the template before depositing the polyelectrolytes (i.e. multiple layers of polyelectrolyte).

[0047] After depositing the poly(ethyleneimine) base layer, different polyelectrolytes may be deposited in an alternating manner. For example, a layer of poly(allylamine hydrochloride) as the first polyelectrolyte layer may be deposited on the poly(ethyleneimine). The template with the deposited poly(allylamine hydrochloride) may be rinsed at least once with deionized water. Poly(sodium 4-styrene sulfonate) as the next polyelectrolyte layer may be deposited on the poly(allylamine hydrochloride). The template with the poly(sodium 4-styrene sulfonate) may be rinsed with deionized water at least once. These steps may be repeated to deposit the poly(allylamine

hydrochloride) and poly(sodium 4-styrene sulfonate) in to form alternating layers of polyelectrolyte. In various embodiments, depositing the polyelectrolytes may include depositing different polyelectrolytes in an alternating manner. The deposition of the base layer and the polyelectrolytes may be carried by any suitable methods, such as dipping (i.e. dip-coating) the template (e.g. with the layers) into a solution containing the respective polymer for the base layer and polyelectrolytes.

[0048] The present method may further include removing the template. As mentioned above, the template may be a sacrificial template, e.g. the PMMA template, that is not present in the oxygen sensor.

[0049] In various embodiments, contacting the dye carrier may include mixing a dye with a particle. The particle may include or may be a hollow porous silica particle or a solid particle that includes a calcium carbonate core having a layer of silica formed thereon. The calcium carbonate core may be removed from the present oxygen sensor. For example, the calcium carbonate core may be removed after the dye is attached to the solid particle. In various embodiments, the present method may further include removing the calcium carbonate core after the dye is attached to the particle to form the dye carrier. The solid particle that includes the calcium carbonate core, which may be denoted as a $\text{CaCO}_3\text{-SiO}_2$ (also referred to as Si-CaCO_3) particle, helps to make the dye carrier structure heavier, which is advantageous for encapsulation in a templated multilayered polyelectrolyte film. The particle may be more rigid and render opaque optical properties of the dye complex, which in turn renders strong light scattering effect. When the CaCO_3 is dissolved away later on, the resultant hollow SiO_2 particle is advantageous in that it is stable in water dispersion, which makes fabrication more convenient without use of organic solvents, and the resultant hollow SiO_2 particle renders a 'transparent' structure having weak light scattering effect.

[0050] In summary, the present oxygen sensor includes a sensing complex, or more precisely, an oxygen sensing complex. The oxygen sensing complex may be, for example, PtTCPP-HSi , wherein PtTCPP is the fluorescent dye and HSi denotes for hollow silica particle, which acts as the dye carrier. The oxygen sensing complex may be in the form of microparticles easily dispersible in water and capable of being maintained as a suspension for a prolonged time (without undesirable agglomeration),

allowing its further inclusion in a hydrophilic matrix, such as a polyelectrolyte multilayered film (with or without an array of chambers for confining the dye carrier).

[0051] Advantageously, the present oxygen sensor is able to sense (i.e. detect) oxygen in water and in gas. The oxygen sensor, e.g. PtTCPP-HSi oxygen sensing complex, may be trapped within a polyelectrolyte multilayer membrane film or inside an imprinted polyelectrolyte having chambers (or referred to as microchambers), which prevents leakage of the dye and dye carrier, in turn protecting the environment and packaged payload from contamination. For the latter, as an example, the oxygen sensor may be configured as a tag and placed in a food packaging containing food. With the dye carrier and dye confined in the multiple layers of polyelectrolyte or chambers in the multilayered polyelectrolyte film, the food does not get compromised.

[0052] The oxygen sensor is made of biocompatible and non-toxic polymers with no direct contact between the dye and packaged payload.

[0053] The oxygen sensor can be configured as an oxygen-sensing film tag that can be easily integrated with any conventional vacuum and modified atmosphere packaging.

[0054] In various instance, tetrakis(4-carboxyphenyl)porphyrin platinum complex (PtTCPP) is used as a non-limiting example of the oxygen sensing fluorescent dye which quenched fluorescence during an oxidation process. The hollow silica (HSi) particles are used as dye carrier to create water dispersible complex.

[0055] Advantageously, the method for forming the oxygen sensor does not require use of complex or uneconomical chemicals. For example, the PtTCPP dye and HSi particles are originally hydrophobic. However, the covalently bonded PtTCPP-HSi complex possesses good dispersibility in water. Thus, the dye can be easily incorporated in a hydrophilic polyelectrolyte multilayer membrane.

[0056] Advantageously, the oxygen sensor can detect oxygen in water. The ability of the PtTCPP-HSi complex to sense oxygen in water is as good as in a gas.

[0057] As mentioned above, the oxygen sensor involves incorporation of the dye and dye carrier in a multilayered film. The HSi particles are very light compared to porous non-hollow silica particles. Thus, the HSi particles have a better stability as a suspension for a longer time than the non-hollow silica particles. The use of PtTCPP-HSi water suspension enables efficient absorption of PtTCPP-HSi onto a charged surface of a polyelectrolyte layer. Besides, the complex does not fall off or detach from

the film surface due to its low weight and can be further sealed with an oppositely charged layer.

[0058] 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,
5 the word “substantially” may be omitted from the definition of the present disclosure.

[0059] In the context of various embodiments, the articles “a”, “an” and “the” as used with regard to a feature or element include a reference to one or more of the features or elements.

[0060] In the context of various embodiments, the term “about” or “approximately” as
10 applied to a numeric value encompasses the exact value and a reasonable variance.

[0061] As used herein, the term “and/or” includes any and all combinations of one or more of the associated listed items.

[0062] Unless specified otherwise, the terms “comprising” and “comprise”, and grammatical variants thereof, are intended to represent “open” or “inclusive” language
15 such that they include recited elements but also permit inclusion of additional, unrecited elements.

Examples

[0063] The present disclosure relates to an oxygen-sensing sensor. For brevity, the
20 oxygen-sensing sensor is herein referred to as “oxygen sensor”, or simply “sensor”. The oxygen sensor may be incorporated to a vacuum packaging or modified atmosphere packaging (MAP) for remote and non-invasive optical signal collection. The vacuum packaging and MAP may be a food packaging. The present sensor may be configured in a system that may include the sensor as a film incorporated with an oxygen-sensing
25 complex and a portable optical scanner.

[0064] The present oxygen sensor, its uses and methods of forming the oxygen sensor, are described in further details, by way of non-limiting examples, as set forth below.

[0065] Example 1: Surface Modification of HSi Particles and Synthesis of PtTCPP-HSi Complex

30 [0066] In order to improve oxygen sensitive PtTCPP loading efficiency, use of mesoporous hollow silica (HSi) was focused on as dye carrier. The mesoporous hollow silica, which has a hollow core and a mesoporous shell, provides considerable surface

area for reactions. The HSi was aminated by (3-aminopropyl)triethoxysilane (APTES). As a non-limiting example of amination treatment, 0.1 g HSi, 3 mL of anhydrous toluene, and 0.1 mL of APTES (with 3% of toluene) were put into a reaction vessel, and shaken overnight. Finally, the aminated HSi was collected by centrifuge. The solid
5 collected was washed two times by 1 mL of toluene and dried. Finally, the solid product was heated at 80°C for 1 hr.

[0067] PtTCPP can be covalently coupled to the surface of the aminated HSi through activation of the carboxylated functional groups of PtTCPP with chemical reagents 1-ethyl-3-[3-dimethyl-aminopropyl] carbodiimide (EDC) and N-hydroxysulfosuccinimide (Sulfo-NHS). In the present example, 20 mg of PtTCPP, 5
10 mL of DMF, 20 mg of EDC, and 20 mg of Sulfo-NHS were mixed for 5 mins in a vessel, followed by addition of HSi (30 mg). The mixture was stirred overnight. Finally, the PtTCPP loaded HSi was collected by centrifuge. The solid product was washed for several times with DMF, and then washed with ethanol, till no color observed in the
15 solution.

[0068] Example 2: Synthesis of PtTCPP-Si-CaCO₃ Complex

[0069] Alternatively, PtTCPP can be loaded on Si-CaCO₃ particles using the similar approach described above in example 1, but silica having undissolved CaCO₃ cores were used instead of hollow silica, obtaining heavy particles which can be easily
20 sedimented and loaded in the multilayered polyelectrolytes.

[0070] After coupling of PtTCPP on the Si-CaCO₃ particles, CaCO₃ can be dissolved using 1 M HCl.

[0071] Example 3A: Membrane Film Sensors Fabrication Using ‘Chamber’ Film

[0072] The ‘Chamber’ film in this example refers to a multilayered polyelectrolyte film
25 having chambers therein. Such chambers may be termed herein ‘microchambers’. Such chambers can be used to house the dye carrier (e.g. PtTCPP-particle complex). To fabricate such a film having chambers, a template approach is used. The template approach involves using, for example, a sacrificial template for forming the chambers in the film. For the purpose of demonstration and not to limit the types of sacrificial
30 template usable, a poly(methylmethacrylate) (PMMA) sacrificial template was first fabricated. To do so, a customized mold (e.g. a silicon mold) was soaked in piranha solution (H₂SO₄:H₂O₂ = 3:1, 140°C) for 30 mins, rinsed with deionized (DI) water and

dried under N₂ gas. The mold was dried in an oven for half a day. Then the mold was salinized using 30 uL of perfluorodecyltrichlorosilane (FDTS) in a desiccator overnight. [0073] Patterns were then imprinted on the PMMA. To do so, the polymers and mold were stacked in the manner as shown in FIG. 2, e.g. silicon wafer / polyethylene (PE) film / PMMA / silanized mold (Si mold) / PE film. Pressure (e.g. 4 MPa) and temperature (e.g. 140°C) were then applied for about 5 mins to let the PMMA flow into the trenches of the mold. The imprint process was completed by cooling the temperature (e.g. to 80°C) and releasing the pressure. Finally, the assembly was demounted and the imprinted PMMA film was detached from the mold. The imprinted PMMA may contain three dimensional structures (i.e. microchambers), for example, pyramidal structures with the sharp edge facing the Si mold. The shape of the microchambers depend on the shape imparted by the Si mold. Other examples of shapes, such as any frustum-shaped, tubular, or cuboidal structures may be fabricated.

[0074] Next, to form the multilayered polyelectrolyte 'chamber' film, dip-coating of various polyelectrolytes was carried out layer by layer. Prior to that, the negatively charged PMMA template film (containing the structures that confer microchambers formation to the layers of polyelectrolyte) may be placed or adhered onto a microscope glass slide using grease glue and then sonicated in water for 5 mins. The negatively charged PMMA template film was then exposed for 15 mins to 2 mg/ml branched poly(ethyleneimine) (PEI) solution (in 2 M NaCl with pH adjusted to 5.5 using 1 M HCl) in order to generate the first anchoring layer (i.e. base layer) with high density of positive charges. Then poly(allylamine hydrochloride) (PAH) solution (2 mg/ml, in 2M NaCl) and poly(sodium 4-styrene sulfonate) (PSS) solution (2 mg/ml, in 2M NaCl) were alternatively dip-coated thereon. For each dip-coating, the duration in a polyelectrolyte dipping solution was about 900 seconds and the fabricated film was rinsed in water for 60 seconds for 3 times. After 60 bilayers (i.e. 60 PAH-PSS bilayers) fabricated, the dip-coating ended with PSS as the final layer. After washing with water, the fabricated 'chamber' film was kept in water. After the multilayered polyelectrolyte film is formed, the PMMA template film may be removed therefrom.

[0075] To illustrate further, one example of the layer-by-layer dip-coating sequence may be as follows.

[0076] (1) To first dip the PMMA template film in the PEI solution and then rinsed 3 times with DI water.

[0077] (2) Next, the PMMA template film coated with PEI may then be dipped into the PSS solution and then rinsed 3 times with DI water.

5 [0078] (3) The PSS coated PMMA template film may then be dipped in the PAH solution and then rinsed 3 times with DI water.

[0079] (4) Repeat steps (2) and (3) until the number of desired layers are formed.

[0080] (5) Dipping in the PSS solution to have a PSS electrolyte layer formed as the final layer.

10 [0081] The various solutions, including some examples of polyelectrolyte dipping solutions for dip-coating are indicated in the table below.

Polyelectrolyte	Concentration	Other Conditions
PAH	2 mg/ml in 2 M NaCl	
PSS	2 mg/ml in 2 M NaCl	
PEI	2 mg/ml in 2 M NaCl	adjusted to about pH 5
PDADMAC	2 mg/ml in 2 M NaCl	

[0082] As an alternative to PAH, poly(diallyldimethylammonium chloride) (PDADMAC) (2 mg/ml in 2 M NaCl) may be used. One example of the layer-by-layer
15 dip-coating sequence using PDADMAC may be as follows.

[0083] (1) Clean patterned glass slide by wiping with acetone and optic grade cleaning wipe.

[0084] (2) Adhere the patterned glass slide onto a microscope glass slide using double-sided tape

20 [0085] (3) To first dip the patterned glass slide in the PEI solution and then rinsed 3 times with DI water.

[0086] (4) Next, the patterned glass slide coated with PEI may then be dipped into the PSS solution and then rinsed 3 times with DI water.

25 [0087] (5) The PSS coated patterned glass slide may then be dipped in the PDADMAC solution and then rinsed 3 times with DI water.

[0088] (6) Repeat steps (4) and (5) until the number of desired layers are formed.

[0089] For each dip-coating, the duration in a polyelectrolyte dipping solution was about 900 seconds and the fabricated film was rinsed in water for 60 seconds for 3 times.

[0090] In this instance, as an example, 8 bilayers of PSS-PDADMAC were formed. After forming the 8 bilayers, the film was left to dry in air. After the multilayered polyelectrolyte film was formed, the glass slide may be removed therefrom.

[0091] Example 3B: Fluorescent Complex Loading into ‘Chamber’ Film

5 [0092] In various examples, the obtained fluorescent complex (i.e. dye carrier) was loaded into the ‘chamber’ film overnight. The dye carrier is already described in examples 1 and 2. The steps are described as follow.

[0093] 1. Prepare a stock solution: SNARF-1-Dextran 1 mg/mL (dissolve 5 mg in 5 ml DI water). SNARF denotes for seminaphtharhodafluor, which is a fluorescent dye that
10 changes color with pH. In various non-limiting instances, SNARF may be used as a proxy for microscopy and not as the oxygen sensing complex.

[0094] 2. Tape down a wet coated sample of the multilayered polyelectrolyte film in a small petri dish (3 cm or 5 cm diameter).

15 [0095] 3. Pour the stock (2.5 to 5 mL) in the petri dish with a thin layer of grease on the edge to be sure that the top cover hermetically closes the dish.

[0096] 4. Hermetically close the dish and wrap with an aluminum foil.

[0097] 5. Put on a horizontal shaker overnight operated with 100 rpm for 24 hrs.

[0098] 6. Remove the sample and clean off excess SNARF-1 from the back with kimwipe (an example of an optic grade cleaning wipe).

20 [0099] 7. Dry the fluorescent loaded film in another small petri dish with grease on the edge and cover with aluminum foil overnight.

[0100] Example 3C: Sealing of Loaded ‘Chamber’ Film

[0101] The following steps were carried out to confine the fluorescent loading in the ‘chamber’ of the film.

25 [0102] 1. Add 2 μ L of DI water on the glass substrate (2 μ L in a 1 cm by 1 cm patterned area).

[0103] 2. Place the PMMA-PEI-(PSS-PAH)₆₀-PSS with the fluorescent SNARF (cut out imprinted area) over glass substrate. The subscript “60” denotes the number of PSS-PAH layer. Herein, PEI forms the base layer on the PMMA template and PSS forms
30 the last layer away from the PEI base layer.

[0104] 3. Place a piece of kimwipe above the multilayered polyelectrolyte film and/or below the PMMA (i.e. between the PMMA and glass substrate).

[00105] 4. “Imprint” at room temperature (e.g. about 30°C) and a pressure of about 10 bars for 1 hr. The “imprint” in this specific instance is the use of the imprinting machine to apply uniform pressure on the multilayered polyelectrolyte film for a prolonged duration to help seal the layers of polyelectrolytes with the dye loaded therein. The use of the imprinting machine may help retain the microchambers’ structure.

[00106] **Example 3D: Template Demolding**

[00107] Two ways of demolding the PMMA template from the multilayered films were demonstrated. The first is to dissolve the PMMA (about half a day) and the other is to mechanically demold the PMMA.

10 [00108] In the first approach, the following steps were carried out.

[00109] 1. Soak in anhydrous toluene for 2 hrs to dissolve the PMMA, swirl if necessary, the PMMA-PEI-(PSS-PAH)₆₀-PSS film may be placed vertically in the anhydrous toluene.

15 [00110] 2. Rinse with clean anhydrous toluene and leave the sensor to dry (cover with aluminum for 2 hrs).

[00111] 3. Clean beakers with acetone, isopropyl alcohol (IPA), ethanol before washing with DI water.

[00112] In the other approach, the PMMA template was mechanically demolded without dissolving by carefully peeling off the film from the template. This approach was demonstrated using 2 cm² templates. Adhesion properties between the multilayer film and template may be considered to perform demolding via this approach.

[00113] **Example 4: Membrane Film Sensor Fabrication Using Screen Printing or Molding Approach**

25 [00114] In examples 3A to 3D above, a template was used so as to form the microchambers in the multilayered polyelectrolyte film. However, in this example, the multilayered polyelectrolyte film was formed without the microchambers. Said differently, the approaches demonstrated in this example may involve a multilayered polyelectrolyte film assembling without using an imprinted template. For example, layers of oppositely charged polyelectrolytes can be deposited in an alternating manner on a flat substrate and the dye carriers can be deposited between the layers from a water dispersion relying on an electrostatic force, instead of having the dye carrier encapsulated in microchambers.

[00115] In this example, the screen printing approach is first described, wherein the dye carrier may be referred to as “ink”. The ink may be first prepared for screen printing of the sensor, wherein the sensor can be in the form of a membrane. The ink that was ready for screen printing included four components: a solvent, a dye, a base (i.e. an alkali), and an emulsifier. The oxygen sensitive complex described above was used as a dye and polyvinylpyrrolidone (PVP) may be used as a polymer matrix. The ink was prepared following the steps described below. The PVP is not suitable for use in the template approach described in examples 3A to 3D.

[00116] 5 mL of ethanol:DI water mixed 50/50% was used as solvent.

10 [00117] 100 mg of sodium dodecyl sulfate (SDS), as emulsifier, was dissolved in the solvent using magnetic stirrer for 1 hr.

[00118] 2.5 mg dry TCPP-HSi was added to the solution and ultrasonicated for 20 mins to break down and prevent any aggregates. Then obtained dispersion was stirred for 4 hrs.

15 [00119] 400 mg of PVP, 30 kDa, was added to the obtained dispersion and stirred for 24 hrs.

[00120] The obtained dispersion was stored for 3 days, during which the dye slightly sedimented (see FIG. 3). As such, the ink dispersion has to be properly shaken before use.

20 [00121] After the ink was prepared, the sensor was fabricated. Sensors were fabricated by molding the ink. The mold was done using glass substrate with attached polyethylene film and a 3M self-adhesive tape was used as a template, wherein different number of layers simulated different thickness of the template. The ink was drop-casted into obtained holes and then excess ink was removed by thin glass slide. The samples were left to dry overnight. The polymer film was removed by peeling off the tape. Obtained samples can be seen in FIG. 4A (sample fabrication) and 4B (printed samples on PE substrate).

25 [00122] In the second approach, samples were prepared using a molding method. A 96 wells plate with its bottom part removed, was used as a mould. The samples were casted on a PE substrate laid on the wells plate. Fabricated samples can be seen in FIG. 5. Samples were fabricated using different amount of ink: 50 mL, 100 mL, 150 mL, and

200 mL. It can be noticed that due to high adhesive properties of PVP, the ink stuck to the surface of the wells.

[00123] Example 5A: Calibration of the Present Oxygen Sensor – Measuring Chamber

5 [00124] The samples were tested in a gas space as well as in dipped in DI water. The level of oxygen was controlled by placing samples in a specially designed chamber equipped with gas inlet and outlet to blow in inert gases like N₂ or Ar to replace O₂. FIG. 6 is a schematic illustration of such chamber. (1) denotes a plastic chamber with a lead to load a sample inside. The container is blacked out to avoid ambient light passing
10 through the walls. The container has a small transparent window positioned under the mounting for the sensing film to measure the film. (2) denotes a gas inlet made of polyvinyl chloride (PVC) tube. (3) denotes a gas outlet to release gas. (4) denotes DI water level. (5) denotes the sample.

15 **[00125] Example 5B: Calibration of the Present Oxygen Sensor – Measuring Oxygen Concentration in Headspace**

[00126] Fluorescent spectra of the samples were measure using Nikon TiRF microscope equipped with Sharmlok monochromator and Andor CCD detector, and 510 nm fluorescent tube (510 nm short-pass excitation filter, 510 nm dichroic mirror, 520 nm long-pass emission filter).

20 [00127] Firstly, samples were measured in a headspace where normal atmosphere was slowly replaced by N₂ gas. It can be seen that fluorescent peak 700 nm is increasing with reducing oxygen concentration in FIG. 7A. Peak ratio at 700 nm and 580 nm was used to calibrate the sensor against the oxygen concentration.

25 **[00128] Example 5C: Calibration of the Present Oxygen Sensor – Measuring Oxygen Concentration in Fluid**

[00129] The measuring chamber described in example 5A was used to measure dissolved oxygen concentration where oxygen concentration was controlled by bubbling N₂ gas through DI water. Obtained fluorescent spectra of the oxygen sensing film can be seen in FIG. 8. In FIG. 8, it is observable that a fluorescent peak at 700 nm, which represents oxygen concentration in headspace, is also responsible for dissolved
30 oxygen concentration and it can be observed that it is rising with reducing oxygen concentration.

[00130] Example 5D: Oxygen Sensor Calibration in Vacuum Chamber

[00131] The fluorescent signal from the oxygen sensor can be obtained using conventional fluorescent spectroscopy, conventional fluorescent spectroscopy equipped with reflectance optical probe, and etc. The oxygen sensor can be configured as a tag as shown in FIG. 9.

[00132] Oxygen sensing tag calibration was performed using vacuum chamber with controlled pressure and one transparent side (the vacuum chamber was provided by Lathercond Technologies Pte Ltd, Singapore). The tag was placed inside the chamber and was measured using the handheld scanner shown in FIG. 9.

[00133] Obtained fluorescent spectra were processed using Python. Here the peak area ratio between 700 nm peak (from 560 nm to 700 nm) and total area and spectrum was used to calibrate the sensors (see FIG. 10).

[00134] Obtained ratios were plotted against oxygen concentration extracted from pressure level inside the chamber (see FIG. 11). The experimental data can be fitted by exponential decay function $x = a \exp(-b y) + c$, wherein $a = 80.806$, $b = 0.004$, and $c = 195,651$. This function can be used to process signal and extract oxygen concentration.

[00135] Example 6A: Monitoring Integrity of Vacuum/MAP Packaged Dry Food

[00136] The described sensing tag and handheld scanner can be used to monitor leaking packages in supply chain along various instances, e.g. (i) starting from the packaging line, rejecting improperly sealed packages and sending them to repack, and down to customer, (ii) monitor slow leaking packages which appeared only after leaving a factory. The present sensor helps to avoid all leaking packages from the supply chain to prevent leaking packages purchased by consumers. The present sensor is demonstrated using vacuum packed rice as one example as shown in FIG. 12.

[00137] Example 6B: Monitoring integrity of Vacuum/MAP packaged meat

[00138] The present sensor was demonstrated for monitoring improperly sealed packages of fresh meat as well as measuring oxygen level inside and oxygen migration through packaging material (see FIG. 13).

[00139] Example 6C: Monitoring Oxygen Migration through Packaging Material

[00140] The present sensor was further demonstrated for not only monitoring improperly sealed packages, but also measure oxygen level inside packages which risen due to natural migration through the packaging material. For example, vacuum

packaged rice (described in example 6A) was used to demonstrate for measurement of oxygen migration inside the package. It is observed that over a course of 3 weeks, the oxygen level reached atmospheric level (also see FIG. 14).

[00141] Example 6D: Monitoring Oxygen Level in Vacuum Insulation Panels

5 **[00142]** The present sensor was demonstrated for measuring oxygen level and checking oxygen leakage of vacuum insulation panels during their exploitation (see FIG. 15).

[00143] Example 7: Summary and Commercial/Potential Applications

[00144] The present oxygen sensor involves microparticle immobilized dye that is dispersible in water, allowing for easy incorporation of the sensing element in various
10 sensor designs. The microparticle immobilized dye can be incorporated into sensing films using various methods, including but not limited to screen printing, embedding in polymeric multilayers, and encapsulation.

[00145] The presently developed microparticle immobilized oxygen sensitive complex and sensing films can be used to monitor the oxygen level in the vacuum and modified
15 atmosphere packages. The developed technology can be used for packaged food, pharmaceutical, electronics helping to reduce waste and improve the supply chain.

[00146] The present sensor, with the handheld sensor, can be operably used in different industries where vacuum and/or oxygen free packaging are used. The industries may include electronics and pharmaceutical industries. Also, the present sensor can be used
20 in laboratories to monitor oxygen level in bioreactors, and to perform environment monitoring in aquaculture farming industry.

[00147] While the present disclosure has been particularly shown and described with reference to specific embodiments, it should be understood by those skilled in the art that various changes in form and detail may be made therein without departing from
25 the spirit and scope of the present disclosure as defined by the appended claims. The scope of the present disclosure is thus indicated by the appended claims and all changes which come within the meaning and range of equivalency of the claims are therefore intended to be embraced.

CLAIMS

1. An oxygen sensor comprising:
a multilayered polyelectrolyte film;
a dye carrier comprising a dye which (i) emits fluorescence in response to a stimuli and (ii) exhibits fluorescence emission inversely proportional to the amount of oxygen present;
wherein the dye carrier is incorporated in the multilayered polyelectrolyte film,
and
wherein the dye carrier comprises a particle covalently attached with the dye.
2. The oxygen sensor of claim 1, wherein the multilayered polyelectrolyte film comprises
a semi-permeable film containing multiple layers of polyelectrolyte absent of hollow chambers, or
a film containing multiple layers of polyelectrolyte having hollow chambers therein arranged in an array.
3. The oxygen sensor of claim 2, wherein the multiple layers of polyelectrolyte comprise poly(allylamine hydrochloride) and/or poly(sodium 4-styrene sulfonate).
4. The oxygen sensor of claim 2 or 3, further comprising poly(ethyleneimine), wherein the multiple layers of polyelectrolyte are formed on the poly(ethyleneimine).
5. The oxygen sensor of any one of claims 2 to 4, wherein the dye carrier is confined within (i) the multiple layer of polyelectrolyte and/or (ii) the hollow chambers.
6. The oxygen sensor of any one of claims 1 to 5, wherein the dye comprises tetrakis(4-carboxyphenyl)porphyrin platinum complex.
7. The oxygen sensor of any one of claims 1 to 6, wherein the particle comprises porous hollow silica particle.

8. The oxygen sensor of any one of claims 1 to 7, wherein the particle comprises mesoporous hollow silica particle.
9. An oxygen sensor film tag comprising:
 - the oxygen sensor of any one of claims 1 to 8;
 - a food-safe barrier film which is arranged to face food in a food package;
 - a transparent protective film arranged distal to the food-safe barrier film; and
 - an adhesive layer for adhering the oxygen sensor film tag to a surface of the food package,wherein the oxygen sensor is arranged between the food-safe barrier film and the transparent protective film.
10. A method of forming the oxygen sensor of any one of claims 1 to 8, the method comprising:
 - providing a template having depressions arranged as an array;
 - depositing polyelectrolytes on the template to form multiple layers of polyelectrolyte;
 - contacting a dye carrier with the multiple layers of polyelectrolyte; and
 - sealing the multiple layers of polyelectrolyte to have an array of hollow chambers defined therein, wherein the dye carrier is confined in the hollow chamber.
11. The method of claim 10, further comprising forming a layer of poly(ethyleneimine) on the template before depositing the polyelectrolytes.
12. The method of claim 10 or 11, wherein depositing the polyelectrolytes comprises depositing different polyelectrolytes in an alternating manner.
13. The method of any one of claims 10 to 12, further comprising removing the template.

14. The method of any one of claims 10 to 13, wherein contacting the dye carrier comprises mixing a dye with a particle, wherein the particle comprises hollow porous silica particle or a solid particle comprising a calcium carbonate core having a layer of silica formed thereon.

15. The method of claim 14, further comprising removing the calcium carbonate core after the dye is attached to the particle to form the dye carrier.