Oxygen Indicator Composed of an Organic/Inorganic Hybrid Compound of Methylene Blue, Reductant, Surfactant and Saponite

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An organic/inorganic hybrid compound consisting of methylene blue, a cationic surfactant and a reductant intercalated into saponite was found to serve as an oxygen indicator that changes color in the presence of oxygen. A mixture of a blue colored dye, methylene blue, a reductant in the form of ascorbic acid or reducing sugar, and cetyltrimethylammonium ion intercalated into synthetic saponite became colorless in an atmosphere having an oxygen concentration of less than 0.1 vol%, and then returned to its blue color as a result of subsequent exposure to air. An oxygen indicator, in the form of a thin film coated on paper prepared by adding a pigment, phloxine B, to the above organic/inorganic hybrid compound, exhibited a pink color at oxygen concentrations of less than 0.1 vol%, and a blue color at oxygen concentrations of higher than 0.5 vol%. In addition, this oxygen indicator exhibited superior photo-fading resistance and storage stability compared with indicators using only methylene blue as the functional dye.

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Introduction

The oxygen molecule is a chemical species that is essential for life and is involved in the most diverse range of chemical reactions and physical phenomena. Since oxygen present in the air allows the growth of molds and other strict aerobic microorganisms and oxidizes nutrients, such as fats and oils, it is one of the primary factors controlling food deterioration.¹ Enormous efforts have been made to develop oxygen sensors and oxygen indicators that can easily detect oxygen gas.²⁻⁵ In contrast to oxygen sensors that are capable of detecting oxygen molecules and converting the detecting information into electrical signals in a quantitative manner, oxygen indicators are defined as devices that enable people to recognize changes in the optical absorbance of organic dyes or pigments according to the oxygen concentration in the form of changes in color in a semi-quantitative or a rather qualitative manner. Thus, from the viewpoint of detection, oxygen indicators can offer several advantages over oxygen sensors due to their immunity from electrical and electromagnetic interferences, durability, small size and their low cost.

Conventional oxygen indicators are known to use methylene blue (methyl thionine chloride (MB)), a dye that reversibly changes its color upon oxidation and reduction, as shown in Fig. 1.^{6,7} Various oxygen indicators, such as ones in the form of a solid for the greater ease of handling,⁸ oxygen-detective ink composites⁹ and their printed materials¹⁰ have been proposed. Actual commercially available tablet-type oxygen indicators

and oxygen indicators in the form of strips of paper coated by an ink composites having an oxygen-detecting function are fabricated to contain an additional red dye or pigment, such as phloxine B, in order to visually emphasize the color change. It enables them to exhibit a blue color in the presence of air and a red color in the absence of oxygen. These oxygen indicators are

Methylene Blue (MB), blue

Leucomethylene Blue (LMB), colorless

Fig. 1 Reduction and oxygen oxidation cycle of methylene blue. Blue-colored methylene blue (MB) is reduced by a reductant to form colorless leucomethylene blue (LMB), and then reverts to MB with its blue color due to oxidation by the oxygen molecule.

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Fig. 2 Schematic structure of saponite.

functional products exhibiting a color change to indicate whether or not the inside of a transparent packaging container is an oxygen-free environment, and are used along with oxygen scavengers to maintain food freshness and to maintain the quality of pharmaceuticals and so forth. However, due to an insufficient resistant character against photo-fading and storage stability of current commercially available oxygen indicators, they become discolored, or suffer a decrease in their color-changing function when stored while exposed to light and at room temperature, and are obliged to be stored in the dark and at low temperature in order to maintain their clear color-changes.

Nano-layered compounds, such as clay minerals, are well known to accommodate various organic molecules within their interlayer. The incorporation of molecules within the interlayer is termed "intercalation". The intercalated complex is called an "organic/inorganic hybrid compound". The intercalated molecules experience a different microenvironment from that in an ordinary solution, and are rather isolated from a bulk-phase solvent when the hybrid compound is dispersed in a solvent. They are, thus, expected to exhibit different characteristics against light irradiation¹¹⁻¹⁵ and high temperature.¹⁶⁻²³ Saponite is a layered clay mineral that has a large cation-exchange capacity, and easily forms organic/inorganic hybrid compounds with cationic organic substances. A schematic structure of the saponite is shown in Fig. 2. Layered clay minerals are also known to form stable compounds with cationic surfactants.^{11-15,24-27} These types of organic/inorganic hybrid compounds are thus expected to be potential candidates that can provide novel microenvironments for the oxygen indicator. From these viewpoints, in this paper we report on the fabrication of a new oxygen indicator using an organic/inorganic hybrid compound consisting of intercalated methylene blue (ionic form, MB⁺), a reductant and a cationic surfactant into the layers of saponite. The highly improved resistant character against light irradiation and storage stability of the oxygen indicator is also reported.

Experimental

Materials

"Sumecton SA" (Kunimine Industries), a synthetic clay saponite, was used as received. The chemical formula of

Table 1 Composition of oxygen-detective ink Liquid A (wt%)

Composition	1	2	3	4
MB	0.10			
MB(0.5)/CTAC(20.3)/saponite(31.7)/MeOH(47.5)		20.00		
MB(1.0)/CTAC(20.2)/saponite(31.5)/MeOH(47.3)			10.00	10.00
Phloxine B	0.05	0.05	0.05	0.05
Ethylene glycol	22.30	22.30	22.30	22.30
Cellulose acetate propionate	6.24	6.14	6.24	5.97
Ethyl acetate	28.08	24.29	20.93	18.04
Isopropyl alcohol	28.08	12.29	20.48	18.04
Fructose	15.15	14.93	20.00	25.60

Sumecton SA is $[(\text{Si}_{7.20}\text{Al}_{0.80})(\text{Mg}_{5.97}\text{Al}_{0.03})\text{O}_{20}(\text{OH})_4]^{-0.77}(\text{Na}_{0.49}\text{Mg}_{0.14})^{+0.77}$. Its specific surface area is $750 \text{ m}^2 \text{ g}^{-1}$, and the theoretical cationic-exchange capacity is 0.997 meq g^{-1} .^{28,29} Ultra-pure water with an electrical conductivity of $0.056 \mu\text{S cm}^{-1}$, obtained with an ultra-pure water treatment system (RO60 (reverse osmosis membrane) + Milli-Q (activated charcoal + ion exchange + precision filtration) system) (Nihon Millipore), was used. "Yupo" polypropylene-based synthetic paper having a thickness of $70 \mu\text{m}$, manufactured by Oji Paper, was used as synthetic paper on which the oxygen-detective ink was printed.

Formation of organic/inorganic hybrid compounds

An aqueous solution of 0.1 N sodium hydroxide was dropped into 20 mL of an aqueous mixture of MB⁺ (0.01 mmol L^{-1}), ascorbic acid or glucose (2.0 mmol L^{-1}), cetyltrimethylammonium chloride (CTAC) (2.0 mmol L^{-1}) and Sumecton SA (0.2 meq L^{-1}) to adjust the pH of the solution to be 11.0, followed by suction filtration (diameter, 35 mm; pore size, $0.2 \mu\text{m}$) of that solution to obtain an organic/inorganic hybrid compound in the form of a clear, blue thin film.

Preparation of oxygen-detective ink pigments

MB⁺ (0.16 g or 0.32 g), CTAC (6.4 g), Sumecton SA (10.0 g) and methanol (15 mL) were mixed using a hybrid mixer (Keyence HM-500) to obtain an oxygen-detective ink pigment.

Composition of oxygen-detective ink

The ink composition of Liquid A (wt%) is summarized in Table 1. In contrast to "Composition 1", containing only MB⁺ as a functional dye, "Compositions 2, 3, 4" contain MB⁺ in the form of an organic/inorganic hybrid compound. In all cases of Compositions 1 through 4, the concentrations of MB⁺ and phloxine B were made to be the same, respectively. In Compositions 1 and 2, the concentrations of the fructose as the reductant were nearly equal to be 15 wt%, while in Compositions 3 and 4, the fructose concentrations were increased to 20 wt% and 26 wt%, respectively. Liquid B contains magnesium hydroxide (43 wt%), cellulose acetate propionate (5 wt%), ethyl acetate (26 wt%), isopropyl alcohol (26 wt%). The composition of the filler was as follows: cyclized rubber resin (10 wt%), magnesium hydroxide (30 wt%), toluene (30 wt%), and cyclohexane (30 wt%). An overprinting agent contained cyclized rubber resin (19 wt%), sodium carbonate (3 wt%), toluene (39 wt%), and cyclohexane (39 wt%).

Analysis

The interlayer distance of the organic/inorganic hybrid compounds was determined by X-ray diffraction measurements

(Rigaku RINT2100). The amount of MB⁺ intercalated into the hybrid compound was determined by the decrease in the absorbance of the MB⁺ mother solution. The composition of the organic/inorganic hybrid compounds was determined by thermogravimetric analysis (Rigaku TAS300/TG8101) and elemental analysis for the total nitrogen (Mitsubishi Chemical TN-10). The absorption spectrum of the oxygen indicators was measured on an ultraviolet-visible spectrophotometer (Shimadzu UV-2500PC) equipped with an integrating sphere unit (Shimadzu ISR-2200).

Evaluation of color changing performance

(1) Anaerobic color changing performance was evaluated by a visual color change of the sample when it was stored at 25°C and 80% RH in a sealed plastic container having a high barrier for gas penetration, together with a commercially available oxygen scavenger (Ageless SA (Mitsubishi Gas Chemical)). A zirconia oxygen concentration meter (Toray LC-700F) was used to monitor the oxygen concentration within the container. The amount of oxygen scavenger and the volume of the container were adjusted so that the oxygen concentration in the container reached 0.1 vol% after about 16 h. (2) The aerobic color-changing performance of the sample was evaluated by opening the plastic container, in which the oxygen concentration was less than 0.1 vol%, to expose it to the air atmosphere. The color change of the sample was visually assessed.

Results and Discussion

Organic/inorganic hybrid compound for detecting oxygen

An organic/inorganic hybrid compound for detecting oxygen was prepared by intercalating MB⁺, a reductant and surfactant, cetyltrimethylammonium ion (CTA⁺), into the layers of saponite. Ascorbic acid or basic glucose was used as the reductant. MB⁺ can easily be intercalated into the layers of saponite by mixing it with an aqueous dispersion of saponite, since it is a cation, itself. The mixture of the MB⁺-intercalated hybrid compound with the reductant, however, did not show any color-change under nitrogen or upon exposure to air. This strongly suggests that the reductants can not react with MB⁺. They might not migrate into the interlayer space where MB⁺ exists. However ascorbic acid and glucose under basic conditions are anionic species. It would be reasonable that these anionic reductants can not penetrate into the interlayer space of the cation-exchangeable clay, saponite, due to the repulsive interaction between the anionic sites of the clay surface and the anionic reductant molecules. To more elegantly incorporate the anionic reductant species within the interlayer of the clay, saponite, on which surface anionic sites are delocalized, we further examined the co-intercalation of a cationic surfactant in the presence of MB⁺ and the anionic reductant species. We have already found that anionic dye molecules can be successfully intercalated within the nano-layered space of niobate³⁰ and saponite by the co-intercalation of cationic surfactants. The cationic surfactant would form an ion association complex with the anionic species, and the resulting complex is incorporated within the interlayer space where the cationic surfactants form a bilayer structure. The hydrophobic interaction between the long alkyl-chains of ionic association compound and the intercalated surfactants may prevail the electrostatically repulsive interaction between the anionic sites on the clay surface and the anionic species. From these viewpoints of strategy, organic/inorganic hybrid compounds were, thus, prepared by co-intercalation with the

Fig. 3 Formation of an organic/inorganic hybrid compound having an oxygen-detection ability. a) Intercalation of methylene blue (MB⁺) being intercalated into saponite and b) co-intercalation of MB⁺ and reducing sugar with cetyltrimethylammonium ion (CTA⁺).

cationic surfactant, CTAC, as shown in Fig. 3. The interlayer distance of the organic/inorganic hybrid compound (including the thicknesses of the layers) slightly increased from 1.31 nm to 1.37 nm when the aqueous dispersion of the clay was mixed with an aqueous solution of MB⁺, and further increased to 2.60 nm by co-intercalation as the result of intercalating CTA⁺. The organic/inorganic hybrid compound was found to consist of 0.056 mol MB⁺, 4.3 mol glucose and 1.2 mol CTA⁺ relative to 1.0 eq of saponite. This organic/inorganic hybrid compound becomes nearly colorless and transparent in a deoxygenated atmosphere having an oxygen concentration of less than 0.1 vol%, and rapidly reverts to its clear, blue color when it is exposed to air, as shown in Fig. 4. These distinct color changes of the hybrid compound was repeatedly observed under repeated exposure to deoxygenated and oxygenated atmospheres, indicating that the blue-colored MB⁺ is reduced into the colorless LMB by the reductant under the deoxygenated atmosphere and is again reverted to MB⁺ by oxygen under air, as expressed in the chemical equation in Fig. 1. A sufficient concentration of glucose as the reductant within the interlayer of the hybrid compound obtained by the co-intercalation method, as described above, renders the system possible to work well as an oxygen indicator.

Oxygen indicator printed with oxygen-detective ink

Composition and preparation of oxygen-detective ink. To fabricate an actual oxygen indicator, an oxygen-detective ink was prepared by blending the resin solution (see Experimental) with an organic/inorganic hybrid compound consisting of MB, CTAC, saponite and fructose; the mixture was then coated onto a strip of paper. A red pigment, phloxine B, was added to the above mixture in order to emphasize a visual color change upon exposure to the deaerated and aerated atmosphere. The oxygen-detective ink was of the two-liquid type. After preparing a liquid in which an organic/inorganic hybrid compound composed of MB, CTAC and saponite, phloxine B and fructose were dispersed in a resin solution (Liquid A (Table 1)), and a liquid in which magnesium hydroxide was dispersed in a resin solution (Liquid B), the two liquids were mixed immediately

Fig. 4 Color change of an organic/inorganic hybrid compound upon exposure to air. The organic/inorganic hybrid compound was composed of MB⁺ (0.056 mol), ascorbic acid (4.3 mol), CTA⁺ (1.2 mol) and saponite (1.0 eq). A semicircular film of the hybrid compound placed on a glass plate became colorless and transparent when subjected to an oxygen-free atmosphere having an oxygen concentration of less than 0.1 vol%, and became blue when exposed to air. Photographs were taken by placing the film of the hybrid compound on a printed letters ("Oxygen Indicator").

just before printing. Polypropylene-based synthetic paper was used as the printed material, which was coated with a filler as the bottom layer, the oxygen-detective ink as the middle one, and an overprinting agent as the top one. The resulting printed material was dried at ambient temperature and cut into short pieces to obtain an oxygen indicator in the form of blue paper strips.

Color-changing performance of the oxygen indicator. The anaerobic and aerobic color-changing performance of the oxygen indicator paper strips fabricated by using different compositions of oxygen-detective ink were evaluated. The results for the different conditions are shown in Fig. 5. When the oxygen concentration inside the containers decreased from 20.9 vol% to 0.1 vol% within 16 h, the oxygen indicators with Compositions 1 and 4 changed their colors from blue to purple, and then turned into pink color after 24 h. On the other hand, the paper strip having Composition 2 was still bluish-purple even after 24 h and one with Composition 3 was purple. A much longer time was required for the paper strips having Compositions 2 and 3 to become pink. All the oxygen indicators that had once become pink returned to their original blue color within 5 min after exposure to air. When this procedure was repeated, the color of these oxygen indicators changed reversibly in response to the oxygen concentration.

Time (h)	0	16	24	48
Oxygen (vol%)	20.9	0.1	< 0.1	< 0.1
Composition 1				
Composition 2				
Composition 3				
Composition 4				

Fig. 5 Color changes of oxygen indicators according to the oxygen concentration. Oxygen indicators fabricated by coating oxygen-detective inks having different compositions (see Table 1) onto synthetic paper were sealed in transparent plastic containers along with an oxygen scavenger, and the color changes of the oxygen indicators were visually observed with the passage of time.

The sensitivity of these oxygen indicators was carefully examined by controlling the oxygen concentration, and it turned out that they exhibited pink color at an oxygen concentration of less than 0.1 vol%, and reversibly exhibited a blue color at an oxygen concentration higher than 0.5 vol%. The color-changing performance of the indicator was not affected by the relative humidity.

Oxygen indicators fabricated by oxygen-detective ink containing MB⁺ intercalated within the organic/inorganic hybrid compound (Compositions 2 and 3) exhibited a slower response for the color change under an anaerobic condition, as compared with the case of containing only MB⁺ (Composition 1). The slower response is thought to be due to a limited diffusion of the reducing agent against MB⁺ within the interlayer space of the hybrid compound. The response, however, was greatly improved to be very fast when the amount of fructose, as the reductant, was increased (Composition 4), which exhibited the best performance as the oxygen indicator among the examined cases, and would promisingly will serve as a commercial oxygen indicator.

Stability and durability of the oxygen indicator

An accelerated photodegradation test and storage-stability test were carried out on the oxygen indicators, as shown in Fig. 6. Paper-strip indicators, fabricated by using oxygen-detective ink with different compositions, were irradiated with visible light of 5000 lx using a fluorescent lamp as the light source in air at 25°C and 60% RH. The photo-fading of MB⁺ was examined by observing any changes in the visible absorption spectrum (diffuse reflectance method). Here, the spectral intensities were expressed by normalizing the intensity at the maximum absorption wavelength of phloxine B. Composition 1 exhibited a relatively large degradation of MB⁺ upon light irradiation, and became purple after 5 h of irradiation. Furthermore, the degradation of MB⁺ during storage for a long time was also significant in the case of Composition 1. It turned into purple after 8 weeks of storage. On the other hand, Compositions 2 through 4 exhibited relatively very little degradation of MB⁺ upon light irradiation or storage for a long time, and maintained their blue color.

Oxygen indicators using MB incorporated within the organic/inorganic hybrid compound were shown to have superior light resistance and storage stability to the case of using MB alone as a functional pigment. The isolated microenvironment of the interlayer space of the clay, saponite, is considered to effectively retard the degradation of MB⁺,

Fig. 6 Changes in the visible absorption spectra of oxygen indicators according to light irradiation time (left) and storage period (right). The visible absorption spectra of oxygen indicators fabricated by coating with oxygen-detective inks having different compositions (see Table 1) onto synthetic paper were observed. The spectral intensities were normalized at the maximum absorption wavelength of phloxine B (543 nm (Composition 1), 555 nm (Composition 4)). Light-irradiation conditions: fluorescent light at 5000 lx, air, 25°C, 60% RH, 5 h. Storage conditions: dark location, air, 25°C, 60% RH, 8 weeks.

mainly by (1) a limited diffusion of oxygen molecules that can oxidatively induce the degradation of MB⁺ through a formation of singlet oxygen generated by quenching of the excited MB⁺ within the interlayer; (2) a strong binding of the cationic MB⁺ on the negatively charged clay surface to prevent the D-D type photo-fading by suppressing the mutual collision of MB⁺ within the interlayer; and (3) a limited diffusion of the third molecule, which might attack MB⁺ either in the excited or ground state to induce degradation.

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