Application of fluorescence in measuring oxygen concentration in packages

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The presence of oxygen in packages of many products, i.e. food products, results in their quality loss, shortening their shelf life and in extreme cases, causes their rottenness. Thus, food manufacturers face a challenge to reduce oxygen in the packages atmosphere in as cheap and effective way as possible, e.g. by using vacuum packing, MAP or oxygen absorbers [1]. However, such actions may fail in bringing about expected effects if there is no confidence whether they have been properly performed. In this case, oxygen concentration inside the package, after its closing, is a measurable indicator. Thus, it is necessary to control oxygen amount in order to verify whether a method used for its removal from the package atmosphere is efficient. There is no information on the processes occurring inside the package without some control over oxygen concentration. Consequently, the state of packed product cannot be evaluated. So far, only gas chromatography or oxygen gauges based on zirconia cells, changing their electric potential depending on the concentration of oxygen present in their surroundings, were used to measure oxygen inside the package. Oxygen can be measured only after prior damage of the package which is the common feature, and at the same time the main disadvantage of these methods. The method is based on gas sampling with a chromatographic syringe, autosampler or a measurement probe with a needle nozzle. However, there can be some leakage caused by piercing the package, which influences the final result of the determination. Additionally, each measurement decreases gas volumes filling the package which reduces the possibility of conducting multiple measurements. A few methods are known which provide for oxygen concentration measurements in the package without its damage. However, regarding various issues, these methods are not commonly used.

This paper presents a new, non-invasive method of oxygen measurements in the package which seems to be the most user-friendly method involving the fluorescence phenomenon.

The method is based on the dependence of the fluorescence quenching of specified substances on oxygen concentration present in their surroundings. Due to this feature, substances can act as specific sensors of oxygen amount [3]. In this case, the fluorescence quenching independent from the presence of gases other than oxygen is a required condition. The quenching mechanism for such a sensor takes place in accordance with reactions where M represents a molecule capable of luminescence and Q is a quenching factor:

$M + h\nu \rightarrow M^*$ photon absorption

$M^* \rightarrow M + h\nu$ luminescence

$M^* \rightarrow M + \Delta$ decay with heat release

$M^* + Q \rightarrow M + Q^*$ dynamic quenching.

Under the conditions of above process, the collision of oxygen molecule (Q) with excited fluorophore (M*) leads to energy exchange and reduction in fluorescence intensity [4]. Then, the energy absorbed by oxygen (Q) is dissipated in the form of heat in short time, and the whole process can be repeated. In the described mechanism, there is no content change in the package atmosphere.

For commercially available oxygen sensors, tri-(4,7-biphenyl--1,10-phenatroline)ruthenium chloride is the most commonly used compound [3]. This is caused by its outstanding stability, durability, high absorption of blue light and induced intensive fluorescence in the scope of red light. This allows of using cheap LED diodes or semiconductor lasers to obtain its excited state. The exemplary measurement system consists of light resources (LED blue diode), an optic cable, detecting equipment (photomultiplier) and a PC with the software to convert the quenching time of fluorescence into oxygen concentration (Fig. 1).

Fig. 1. The exemplary system for oxygen content measurement using the fluorescence phenomenon [2]

During the measurement, ruthenium complex is being excited by the radiation emitted by LED blue diode. A short blue light puls is absorbed by the complex. Then, the complex emits the radiation at maximum 600 nm (Fig. 2A.) The mean fluorescence lifetime is the time between photon absorption and the emission of photon of another colour. For this ruthenium complex, it is ca. 5 microseconds. However, if there is oxygen in the system, fluorescence is quenched (Fig. 2B). During the collision of oxygen molecules with the complex molecules, the energy exchange preventing photon emission is observed. Such a process is known as dynamic quenching which causes the decrease in fluorescence lifetime proportional to the oxygen concentration (partial pressure) in the surrounding atmosphere. The fluorescence almost disappears at extremely high concentration of oxygen.

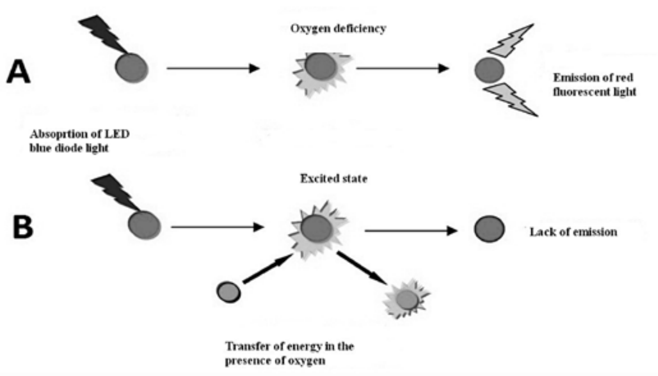
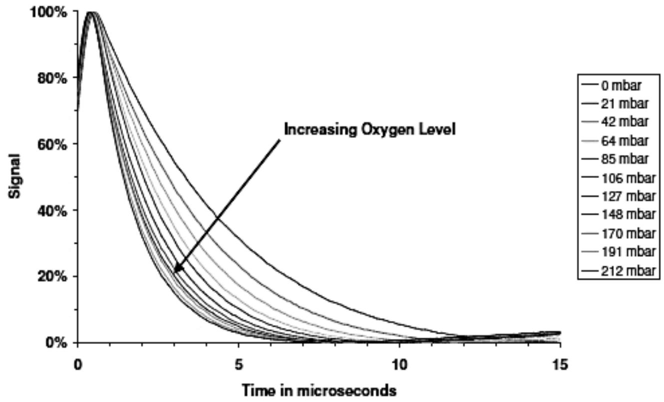


Fig. 2. Fluorescence process for ruthenium complex at oxygen deficiency (A) and its presence (B). Source: own work based upon materials from Oxysense company

Under standard conditions, the lifetime drops from 5 microseconds in the oxygen-free atmosphere at oxygen partial pressure of 0 mbar to ca. 1 microsecond in the atmospheric air for which oxygen partial pressure is 212 mbar. The relations of luminescence time to oxygen partial pressure are described by the Stern-Volmer equation [3, 5] and they are presented on the graph 1.



Graph 1. Quenching time of fluorescence with relation to oxygen partial pressure. Source: own work based upon materials from Oxysense company

Knowing the fluorescence lifetime, the intermediate values of oxygen partial pressure and then its percentage concentration can be determined. This allows for the measurement system calibration using only two points corresponding to specified oxygen partial pressures. In practice, two calibration gases are used. Inert gases free of oxygen (e.g. nitro gen 5,0 or argon 5,0) are used as the zero standard for oxygen concentration, whereas the gas mixture containing the specified oxygen content (e.g. 10% oxygen + 90% nitrogen, 20% oxygen+90% argon) is used as the standard for oxygen high concentration. Oxygen content in the mixture with other inert gas depends on the scope of planned measurements.

On the market, there are available commercial systems for detecting oxygen amount which apply the above method. The examples of such systems are, inter alia: Oxysense GEN III 300 and 5000 systems of OxySense® company and OpTech™ 0_2 Platinum system of MOCON company. They have compact dimensions which enable to move them easily to test sites. The readiness for the measurement involves just plugging the computer into the supply network through the USB connector and starting the designated software, which calculates percentage concentration of oxygen on the basis of registered quenching time of fluorescence. The device emitting excitation light and registering the induced fluorescence is the fundamental part of the instrument. To make the measurement of oxygen concentration in packages easier, these instruments are equipped with an optic cable to transmit the excitation light and the light emitted from the sensor inside the package into the system measuring the intensity and quenching time of fluorescence.

Additionally, there is a temperature sensor at the end of the optic cable which allows for the compensation of temperature impact on the fluorescence time, so the measurements can be conducted at different temperatures of the surroundings and the tested package. Additional heat sources should be avoided because, as observed in conducted tests, these can significantly affect the measurement result.

The tests conducted in Faculty of Food Commodity Science with the application of OxySense® system show that the systems involving fluorescence to measure oxygen in the package atmosphere produce very promising results in the scope of determining oxygen content in the package and they can become an ideal alternative for traditional methods of oxygen concentration measurement discussed in the beginning. They provide almost an immediate reading of oxygen concentration and unlimited number of measurements per one package (this is particularly important in storage tests). Depending on needs, the measurements can be conducted with accuracy up to 0.1 or 0.01% of oxygen. If the tested packages (and sensors containing ruthenium complex) are continuously exposed to daylight, the regular calibration of the system (at least once a week) provides the best results. This is related to a minor change in the fluorescence properties of ruthenium compound under the influence of visible light. The measurements can be conducted in the packaging, regardless of its construction design. The material of the package is to be transparent, which is the only condition and at the same time limitation, of the presented method. The packages made from plastic materials (inter alia, polyethylene, polyamide, polyester and laminates or combinations thereof) in the form of bags and bottles as well as glass packages, i.e. bottles and jars were used in the tests. For plastic packages, the type of material and its thickness did not have any effect on the measurement results and the tests were conducted for materials with the thickness from 15 to 200 μm. An attempt to determine oxygen content in beer bottles made from brown polyethylene terephthalate (PET) was also conducted. The calibration through the tested material should be carried out regarding its colour impact on sensor excitation and emission.

For glass packages, it is important to conduct the measurement through a measurement probe placed as close to the package wall, and the optic sensor placed inside, as possible. Moving the measurement probe away at a distance longer than 5mm from the package surface caused a significant change in the measurements results. The majority of glass packages have cylindrical cross-sections so a part of the excitation and emitted light could be diffused and did not enter the analysing instrument. The measurements of oxygen concentration can be also carried out in the packages made of coloured glass. The positive results were observed both for the most commonly used glass in the packaging industry, i.e. green glass, light brown glass and less often used blue glass (e.g. for the production of wine bottles). However, it should be emphasized that the oxygen concentration measurements are not possible to be performed in the packages made of dark brown glass. In this case, the obtained results were completely incidental.

The non-invasive nature allowing for the measurement without damaging the package is the best advantage of the discussed method. Additionally, none reacting (during the measurements) substrate is consumed which allows for the multiple measurements in the same package. The sensor surrounded with hydrophobic polymer is very stable and resistant to unfavourable external factors, e.g. fats, high temperature, moisture, which property considerably extends the scope of its application. Short time of reaction providing a very quick determination of oxygen content in the package is the next asset. These advantages predispose the discussed method to monitor the packaging processes under modified atmosphere MAP and control tightness of these packages. Also, the possibility of using this method to determine the level of oxygen permeability through the packaging materials and packages in accordance with the methodology approved by American Society for Testing and Materials [6] shall be emphasized. This parameter is one of the most important values which characterize the packaging materials considering their suitability for packing food products. This allows for a proper selection of packaging materials for a specified product and the applied technique for its packaging. The attempts intended to apply the discussed method in medicine, for diagnostic purposes [7, 8], are undertaken. Also studies on future application of fluorescence and optic sensors to determine the concentrations of other gases, e.g. carbon dioxide [9] are conducted.

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Wojciech KOZAK – Ph.D (Eng), graduated from Faculty of Food Commodity Science, Poznań University of Economics (1999). His doctoral thesis on active packages He obtained at Department of Food Commodity Science (2005) was honoured by the decision of the Rector of Poznań University of Economics. Nowadays, he is working at Department of Food Commodity Science at Poznań University of Economics. Scientific interests: active and intelligent packages, advanced materials, automatic identification of products. He is an author of many publications in national and foreign journals, papers and conference communications.

Conference „On the RACH road"

The International Conference **On the REACH road** is organized by the Ministry of Economy, in cooperation with the Bureau for Chemical Substances and the Polish Chamber of Chemical Industry. As the year 2011 was designated by UN as the International Year of Chemistry and 2011 is also the year of celebration of The 100th Anniversary of Maria Skłodowska-Curie Nobel Prize in chemistry - The Conference was appointed as one of the activities undertaken to celebrate the IYC 2011, MSC-100 as well as the 5th Anniversary of the adoption of the REACH regulation.

The conference examines the five years period of REACH implementation as a step towards innovation management and safe use of chemical substances. The aim of the conference is also to assess the industry and policy makers activities on both European and global scale.

This two-day conference will take participants through the following topics:

- Industry experience with REACH (including the situation after the first phase of registration and the SME situation before the second phase of REACH registration)
- To REACH review process (including possible ways of future development of the regulation's provisions)
- Activities undertaken on order to improve the innovation and competitiveness of the chemical industry
- Influence of the REACH regulation of the other international activities in respect of strategic management of chemicals.

The Conference is to be held in Warsaw, Poland, 23-24 November 2011. The event will provide a dynamic environment for information exchange and will bring together the representatives of governmental institution, non-governmental organizations, industry, scientists and chemical experts across Europe and from around the word. It will also provide a great opportunity for delegates to establish business relations for future collaboration on the global, chemical market.