



Standard Test Method for Oxygen Headspace Analysis of Packages Using Fluorescent Decay¹

This standard is issued under the fixed designation F 2714; the number immediately following the designation indicates the year of original adoption or, in the case of revision, the year of last revision. A number in parentheses indicates the year of last reapproval. A superscript epsilon (ϵ) indicates an editorial change since the last revision or reapproval.

1. Scope

1.1 This test method covers a procedure for determination of the oxygen concentration in the headspace within a sealed package without opening or compromising the integrity of the package.

1.2 This test method requires that chemically coated components be placed on the inside surface of the package before closing.

1.3 The package must be either transparent, translucent, or a transparent window must be affixed to the package surface without affecting the package's integrity.

1.4 As this test method determines the oxygen headspace over time, the oxygen permeability can easily be calculated as ingress per unit time as long as the volume of the container is known.

1.5 The values stated in SI units are to be regarded as standard. No other units of measurement are included in this standard.

1.6 *This standard does not purport to address all of the safety concerns, if any, associated with its use. It is the responsibility of the user of this standard to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.*

2. Summary of Test Method

2.1 Chemically coated components (dots) are affixed to the inside surface of the package to be tested.

2.2 The package is gas flushed to a reduced level of oxygen either manually or by subjecting the package to a filling operation.

2.3 A pulsing light source is directed through the package at the chemically treated dot (the package must be transparent, translucent or contain a window through which the light can pass).

2.4 The fluorescent response from the dot is monitored and the decay rate determined.

2.5 The internal oxygen content of the package is determined by comparing the measured decay rate to the decay rate observed with known oxygen concentrations.

3. Significance and Use

3.1 The oxygen content of a package's headspace is an important determinant of the packaging protection afforded by barrier materials. The package under test is typically MAP (modified atmosphere packaging) packaged.

3.2 Oxygen content is a key contributor to off-flavors and spoilage of various products, such as chemicals, food and pharmaceuticals.

3.3 The method determines the oxygen in a closed package headspace. This ability has application in:

3.3.1 *Package Permeability Studies*—The change of headspace composition over a known length of time allows the calculation of permeation. Since the headspace oxygen is measured as a percentage, the volume of the container's headspace must be known to allow conversion into a quantity such as millilitres (ml) of oxygen. The use of this approach to measure permeation generally applies to empty package systems only as oxygen uptake or outgassing of contained products could affect results.

3.3.2 *Leak Detection*—If the headspace contains more oxygen than expected or is increasing faster than expected, a leak can be suspected. A wide variety of techniques can be employed to verify that a leak is present and to identify its location. If necessary or of interest, a leak rate may be calculated with known headspace volume and measured oxygen concentration change over time.

¹ This test method is under the jurisdiction of ASTM Committee F02 on Flexible Barrier Packaging and is the direct responsibility of Subcommittee F02.40 on Package Integrity Test Methods.

Current edition approved Aug. 1, 2008. Published August 2008.

3.3.3 *Efficacy of the MAP Packaging Process*— If the headspace oxygen concentration is found to be higher than expected soon after packaging, the gas flushing process may not be working as well as expected. Various techniques can evaluate whether the MAP system is functioning properly.

3.3.4 *Storage Studies*—As the method is non-destructive, the headspace can be monitored over time on individual samples to insure that results of storage studies such as shelf life testing are correctly interpreted.

4. Discussion

4.1 Oxygen sensing based on fluorescence is well established. The typical indicators used are ruthenium complexes and porphyrins both of which are compatible with light emitting diodes (LEDs). In one oxygen sensitive coating, tris (4,7 biphenyl 1,10 phenanthroline) ruthenium chloride is used due to its stability, long lifetime, and strong absorption between 400 nm and 500 nm in the blue region of the spectrum. The absorption peak is compatible with high brightness blue LEDs or blue semiconductor lasers. The emission peak is at 600 nm in the red region of the spectrum and is detected by a photomultiplier tube or a photo detector to offer the flexibility of a large dynamic range and fast response time. The ruthenium complex is immobilized in a highly chemically resistant substrate.

4.2 The principle of fluorescence quenching is based on the excited state characteristics of a specific dye. Dynamic quenching is the transfer of energy from a fluorescent dye in its excited state to oxygen in the surrounding medium. The energy consumed by oxygen will be dissipated as heat after a short time and the whole process can repeat itself indefinitely without consuming oxygen.

4.3 The ruthenium complex is excited with blue light from an LED. Short pulses of blue light from the LED are absorbed

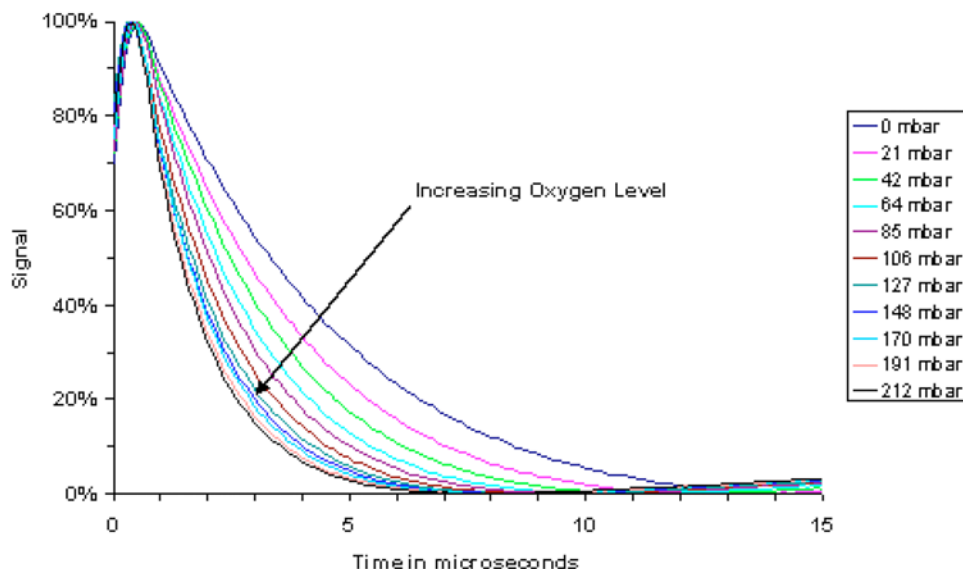
by the ruthenium complex. In the absence of oxygen, the ruthenium complex will emit light in the red region of the spectrum. The average time between the absorption of the blue photon and the release of the red photon is called the fluorescence lifetime. The fluorescence lifetime of the ruthenium complex is about 5 μ s. However, if oxygen is present, the fluorescence is quenched. This occurs when oxygen molecules collide with the excited ruthenium molecules. During the collision, energy is transferred from the ruthenium to the oxygen, preventing emission. This process is called dynamic quenching, and it results in a decrease in the fluorescence lifetime proportional to the oxygen partial pressure. The fluorescence lifetime will decrease from 5 μ s in an oxygen free environment (for example, nitrogen) to 1 μ s in ambient air (see Fig. 1). The most important aspect of using quenching for oxygen detection is that neither the oxygen nor the sensor is consumed during a measurement.

5. Interferences

5.1 The presence of certain interfering substances in the headspace may, in theory, give rise to incorrect readings. Normal headspaces in empty or filled packages have not been found to be problematic. Relative humidity in that headspace also has shown to not cause interferences.

5.2 The temperature of the package, when tested, needs to be measured.

5.3 It is recommended that calibration, described below, of the chemically treated dots be conducted on packages containing known oxygen concentrations as close to the level to be experienced in actual tests. If the calibration is carried out at levels far different than actual levels, the results may show less precision than predicted in the precision and bias statement below.



NOTE—The fluorescent lifetime lies between 1 μ s and 5 μ s.

FIG. 1 Relative Fluorescence Signals (I/I_0) after Illumination of a Short Blue Pulse, Quenched by Different Oxygen Pressures in Air of 20°C

6. Apparatus

6.1 *Chemically Treated Components (aka “dots”)*—Coated substrates of glass or flexible clear plastic have been found to be satisfactory. A fluorescent dye polymer is deposited on one side of the substrate.

6.2 *Adhesive* is used to attach the non-coated side of the dot to the inside of the package. Silicone rubber adhesive has been shown to be satisfactory. Other adhesives and double-sided tape will work as well. No adhesive has yet been identified which interferes with the fluorescence of the dye as long as the adhesive is sufficiently translucent.

6.3 *Light Source* producing sufficient energy in the appropriate wavelength to activate the fluorescent dye. The light energy is pulsed to allow determination of the decay rate.

6.4 *Light Detector* with associated electronics, which is able to determine the decay time of the fluorescence.

6.5 *Computer System* to compare the measured fluorescent decay rate of packages under test to packages containing known oxygen concentrations and present the results as oxygen concentration.

7. Reagents and Materials

7.1 The coating used is described in Section 4. Other complexes may be used but their ability to measure oxygen must be demonstrated.

8. Precautions

8.1 Temperature and relative humidity are critical parameters affecting the measurement of oxygen permeation. If this headspace technique is to be used to calculate the oxygen transmission rate of packages, careful temperature and relative humidity control can help to minimize variations due to environmental fluctuations. The average conditions and range of conditions experienced during the test period shall both be reported.

8.2 Temperature of the chemically coated dot has an effect on the observed decay rate. Care must be taken to ensure that the dots temperature is known and controlled to $\pm 0.1^\circ\text{C}$ at the time that the measurement is made.

9. Sampling

9.1 A statement as to the number of samples to be tested is beyond the scope of this test method.

10. Test Specimens

10.1 The test specimens can take many forms and include any sealed package which contains a headspace containing a gas or liquid.

10.2 The test specimen must be sufficiently translucent to allow the coated dot to be accessed. Alternatively, a window can be added to the package to allow access, but the design of such window is beyond the scope of this test method.

11. Conditioning

11.1 No conditioning is normally necessary except the need to maintain temperature control of the samples as discussed elsewhere in this test method.

12. Procedure

12.1 *Calibration*—Prior to testing, the fluorescent dots and the complete measuring system are to be calibrated.

12.1.1 Fluorescent dots are affixed to the inside of a rigid package, flexible package, or a calibration fixture using adhesives described above. The package or fixture is sealed and flushed thoroughly with gases containing certified levels of oxygen above and below the expected level of the packages to be tested. As the package is flushed through a small opening, it is monitored for indicated oxygen level. When the level does not change for 1 min and the package or fixture has received at least 20 times the headspace volume in flushing gas, the reading can be considered final and the reading entered into the computer based on the manufacturer’s instructions.

12.1.2 The instrument manufacturer’s instructions are followed to test a calibration gas containing below the oxygen level to be tested. Normally, this would be nitrogen, certified to contain no oxygen. The value is entered as instructed.

12.1.3 The instrument manufacturer’s instructions are followed to test a calibration gas containing above the oxygen level to be tested. Normally, this would be nitrogen containing a certified level of oxygen no higher than 3 times the expected level of oxygen in the package to be tested; that is, if the package to be tested is expected to contain 2 % oxygen, then a calibration gas of 6 % would be acceptable.

12.1.4 Calibration using the calibration factors supplied by the instrument manufacturer can be satisfactory, but care must be taken to ensure that the dots have been calibration in the oxygen concentration range of interest as discussed above.

12.2 *Testing*—A previously prepared, sealed, and flushed package of unknown oxygen concentration is measured by a test system calibrated as described above.

12.2.1 Dots that have been affixed within the package of unknown oxygen concentration are exposed to the calibrated, fluorescent system in accordance with the manufacturer’s instructions.

12.2.2 The value indicated by the instrument is noted. The value indicated can be expressed in percent oxygen, partial pressure oxygen, or ppm oxygen.

12.2.3 The environment of the dots can be the gaseous headspace of the package or within a liquid depending on the test design and the information sought.

13. Calculation

13.1 The computer system will do the calibration for the user using the Stern-Volmer equations to convert the decay time into partial pressure of oxygen. The equations use the previously determined response of the system to the calibration gases employed above.

13.2 The red fluorescent signal has a certain delay in rise and decay depending on the oxygen partial pressure ($p\text{O}_2$). This effect is shown in figure below. The typical fluorescent lifetime τ varies between 1 μs in ambient air ($p\text{O}_2 = 212$ mbar at sea level) and 5 μs in zero oxygen.

13.3 From the signal the fluorescence lifetime can be derived. The mono-exponential fluorescence decay can be described by:

$$\frac{I}{I_0} = \exp\left(\frac{-t}{\tau}\right) \quad (1)$$

where:

- I = fluorescence intensity at a certain time,
- I_0 = fluorescence intensity at the start of the decay (in this case on $t = 1 \mu\text{s}$),
- t = time in μs , and
- τ = fluorescence lifetime or time constant (TC).

13.4 The software calculates the time constant τ from a mono-exponential least squares fit of the fluorescence signals generated by the ruthenium in the coated dot, and from the time constant the oxygen concentration is calculated. The relationship between the oxygen partial pressure and the measured fluorescence lifetime (time constant) is given by the Stern-Volmer Equation:

$$\frac{\tau_0}{\tau} = 1 + K_{SV} \cdot pO_2 \quad (2)$$

where:

- τ = time constant at current oxygen concentration,
- τ_0 = time constant in the absence of oxygen,
- K_{SV} = Stern-Volmer constant, and
- pO_2 = oxygen partial pressure.

13.5 This linear Stern-Volmer equation is transposed in the software by Eq 3:

$$\frac{1}{TC} = A \cdot pO_2 + B \quad (3)$$

where:

- TC = time constant at current oxygen concentration in μs (τ in Stern-Volmer equation),
- pO_2 = oxygen partial pressure in mbar,
- A = slope of the Stern-Volmer line, and
- B = intercept of the Stern-Volmer line.

13.6 The calibration process determines the slope and intercept (dA and dB) of the Stern-Volmer line for a low and a high oxygen concentration.

14. Report

14.1 The oxygen concentration (expressed as percent, partial pressure or parts per million) is reported for each sample tested.

14.2 The environment in which the test was conducted.

14.3 The lot number of the dots used and the calibration factors are reported.

15. Precision and Bias

15.1 An interlaboratory study was conducted with 6 laboratories participating. Seven samples were prepared at each of three levels of oxygen concentration (approximately 0.04, 1.02, and 5.05 % oxygen). Each lab tested each sample three times. The repeatability and reproducibility was computed for each sample. The results were similar enough at each level to permit pooling the repeatability and reproducibility standard deviations by level. **Table 1** summarizes the results. More complete results and analyses are provided in a research report.²

16. Keywords

16.1 fluorescence; headspace analysis; oxygen transmission rate; permeability; permeation; residual oxygen

² Supporting data have been filed at ASTM International Headquarters and may be obtained by requesting Research Report RR: F02-1026.

TABLE 1 Precision by Level

Level	Average	s_r	s_R	r	R
Low	0.039	0.006	0.010	0.017	0.029
Medium	1.024	0.009	0.013	0.025	0.038
High	5.050	0.023	0.058	0.063	0.163

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