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Yusufu, D., & Mills, A. (2019). A Colourimetric Vacuum Air-Pressure Indicator. *The Analyst*, *144*(20), 5947-5952. https://doi.org/10.1039/c9an01507h

Published in:
The Analyst

Document Version:
Peer reviewed version

A Colourimetric Vacuum Air-Pressure Indicator

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Abstract

A colourimetric vacuum air pressure indicator is described, based on the very low level of CO_2 in air. The indicator uses the pH indicator dye, *ortho*-cresolphthalein, OCP, which is violet coloured in its deprotonated form and colourless when protonated. When the violet coloured OCP anion is ion-paired with the tetrabutylammonium cation, the product is readily dissolved in a non-aqueous solution containing the polymer ethyl cellulose to create an ink which, when cast and allowed to dry, responds to levels of CO_2 well below that in air, *i.e.* << 0.041%; the indicator's halfway colour changing point is at 0.062 atm of air at 22 ^{o}C, which is interesting in that in food vacuum packaging the pressure in the pack is usually ca. 0.04 atm. The indicator can be used as a *qualitative* and quantitative indicator of vacuum air pressure. The latter requires the use of digital photography, coupled to RGB colour analysis, in the analysis of the indicator's colour. As with most CO_2 indicators, the indicator's response is temperature sensitive, with $\Delta H = 78 \pm 5$ kJ mol^{-1}. The indicators 90% response and recovery times to a cycle of vacuum and air were 16.2 and 2.7 min, respectively. The efficacy of the indicator as a vacuum-package integrity indicator for food packaging is illustrated and other potential applications are discussed briefly. This is the first reported example of an ink-based, inexpensive vacuum air pressure indicator.

Keywords: indicator; vacuum, packaging, carbon dioxide, low pressure, colourimetric

1. Introduction

Vacuum packaging (VP) is a method of packaging which removes air from the package prior to sealing and, amongst other things, is commonly used in wholesale and retail food packaging (especially of: fresh and processed meat and fish, cheese, chocolate, sweets and many different dried goods, such as seaweed and rice); after packaging, the pressure inside the pack is typically ca. 0.04 atm.[1] VP can extend the shelf-life of foods by days, although more often weeks and, in some cases, months.[1] In addition, vacuum packagers can be found in most household and restaurant kitchens because of their low cost and ease of use. VP is also used for pharmaceutical and medical products, electronic components, such as: semiconductors, microchips, memory chips, sub panels, motherboards, PLC's and RAM,[2] and coins and collectables.[3] Interestingly, despite its widespread use, there is no quick, simple, inexpensive method for measuring the vacuum pressure inside such packages and so no routine way to assess package integrity after VP. As a consequence, VP quality control is usually limited to periodic sampling of the package line by the packager, often as infrequently as one in every 300-400 packages, and there is little or no subsequent testing of package integrity as the package makes its way to the retailer and then consumer.[1]

It has been demonstrated that it is possible to use reversible luminescence-based oxygen indicators to assess the vacuum level in VP,[4, 5] but these utilise relatively expensive (typically \$4 – 30 each[6]) *luminescent* indicator 'dots' or strips and costly excited-state lifetime measuring equipment.[6] As a consequence, the use of oxygen indicators in food packaging for example is largely limited to packaging research.[4, 5] Although a number of different colourimetric O_2 indicator strips have been reported, mainly based on redox indicators,[7] they are all irreversible, as noted by Wang and Wolfbeis in their seminal review on O_2 indicators and, as a consequence, cannot be used for monitoring O_2 levels quantitatively and so cannot be used for monitoring vacuum air pressures.[8]

As a consequence, there is a real need for an inexpensive, quick, easy to use, *reversible* colour-based vacuum air pressure indicator which allows both a: (i) *qualitative* assessment of vacuum package integrity by eye and (ii) *quantitative* assessment of the pressure inside the package, by digital photography and colour analysis App. The former feature will reassure the consumer regarding packaging integrity and absence of tampering, whilst the latter will improve quality control along the distribution chain from packager to retailer.

The novel approach presented here towards generating a colour based vacuum air pressure indicator is through the detection of the CO_2 that is in air. Obviously, there is not much CO_2 in air, with the average level of CO_2 in air being ca. 411 ppmv (*i.e.* 0.041 %), but what there is could be used to provide a measure of the ambient air pressure using a very sensitive CO_2 indicator. It is assumed here that the vacuum-packaged product, be it a food stuff, medical product *etc*., does not itself alter the CO_2 level in the package. Thus, this new vacuum air-pressure indicator would not be appropriate if the product respired, such as in the case of vacuum-packed meat for example.[6] However, as we shall see, it would be appropriate for non-respiring food stuffs, such as most dry goods.

In the early 90's this group developed a number of different CO_2 indicators derived from solvent-based inks that contained an ion-pair of a pH indicating dye anion, D^-, with a lipophilic quaternary ammonium cation, Q^+, such as the tetraoctyl ammonium cation, so that the ion-pair's formula was: $D^-Q^+xH_2O$;[9, 10] note: like many such ion-pairs, $D^-Q^+xH_2O$ retains a few molecules of water even in non-aqueous solvents. Due to the phase transfer nature of the ion-pair, $D^-Q^+xH_2O$ readily dissolves in a solvent-based ink and when allowed to dry produces a coloured, water-insoluble plastic film that responds to CO_2, as if the dye were dissolved in water. The key process can be summarised as follows:

$$D^-Q^+xH_2O \;+\; CO_2 \;\rightleftharpoons\; HCO_3^-Q^+(x\text{-}1)H_2O.DH \qquad (1)$$

Colour **A** Colour **B**

where, $D^-Q^+xH_2O$ and $HCO_3^-(x\text{-}1)H_2O.DH$ are the deprotonated (D^-) and protonated (HD) forms of the pH-indicating dye, D. It follows from the above that

$$R = [HD]/\,[D^-] = \alpha\times\%CO_2 \qquad (2)$$

where R is a measure of the transformation of the dye from the deprotonated to protonated form due to the presence of CO_2, [HD] and $[D^-]$ are the concentrations of $Q^+HCO_3^-.HD.(x\text{-}1)H_2O$ and $Q^+D^-.xH_2O$, respectively and α is a proportionality constant (units: $\%^{-1}$), which provides a measure of the sensitivity of the CO_2-sensitive optical sensor under test. Most importantly here, note that it can be shown that the value of α depends upon, amongst other things, the value of the pK_a of the dye, increasing with increasing pK_a, and the level of additional base present, $OH^-Q^+xH_2O$, decreasing with increasing $[OH^-Q^+xH_2O]$.[11] As a consequence, the sensitivity of the CO_2 indicator can be varied, *i.e.* tuned, by using different pH-indicating dyes with different pK_a values.[11] Solvent based CO_2-sensitive inks and films dominate the field since, unlike their water-based counterparts, they dry very quickly, and so are more conducive to printing, are not prone to dye leaching by water and largely insensitive to changes in humidity.

Following on from the initial work of this group, many different CO_2 indicators have been reported,[12-15] using the same ion-pair technology, but with different dyes, phase transfer agents (often tetrabutyl ammonium hydroxide, TBAOH) and encapsulating polymers (such as ethyl cellulose (EC), silicone and poly vinyl alcohol). However, in almost all cases, this work has focussed on the detection of super-ambient levels of CO_2, most notably: ca. 5% for the detection of CO_2 in breath, as exemplified by the Easy Cap II® detector[16] and > 10% for the detection of CO_2 in modified atmosphere packaging (MAP), as in Insignia Technologies' After Freshness Indicator.[17, 18] 1-5% CO_2 responding ion-pair indicators usually employ moderately high pK_a dyes, such as *meta*-cresol purple (MCP; pK_a = 8.28) or Cresol Red (CR; pK_a = 7.95), whereas >10% CO_2 responding indicators use pH-dyes with a lower pK_a, such as Phenol Red (PR; pK_a = 7.52). It follows that in order to make a super-sensitive CO_2 indicator for vacuum air pressure work, a dye with a much higher pK_a is required.[11] For this purpose, here we use *ortho*-cresolphthalein (OCP), which is violet coloured in its deprotonated (D^-) anionic form and colourless in its protonated neutral lactone (*i.e.* DH) form and has a pK_a of 9.32. Using such an indicator, this paper describes the preparation and characterisation of the first colourimetric vacuum air pressure indicator.

2. Experimental

Materials

Unless otherwise stated, all chemicals were purchased from Sigma Aldrich in the highest purity available. All solutions were prepared fresh, and all aqueous solutions were made up using double distilled and deionised water. The Sigma Aldrich safety data sheet for OCP provides useful details regarding the handling and disposal of this dye and, under the heading 'toxicological information' notes that 'no component of this product present a levels ≥ 0.1% is identified as probable, possible or confirmed human carcinogen by IARC'.[19]

The super-sensitive CO_2 ink containing OCP was prepared as follows: 0.1 g of *ortho*-cresolphthalein (the pH indicator dye), 2 g methanol (MeOH), 5 ml tetrabutylammonium hydroxide (TBAH) 1M in methanol (the base, $OH^-Q^+xH_2O$, which ensures the dye is converted into a lipophilic, deprotonated ion-paired form, $D^-Q^+xH_2O$, and which provides excess base and so additional control of the value of α)[11], 0.5 ml tributyl phosphate (TBP, the plasticiser which aids the rate of diffusion of the CO_2 into and out of the indicator film)[9,20] and 5 g of an ethyl cellulose (EC) solution, comprising 10 g of EC in a 80/20 (v/v) mix of toluene and EtOH, (EC; the water insoluble polymer encapsulation medium which prevents dye leaching if any water is present)[21] were mixed together in a 30 ml jar and stirred for 2 hours using a magnetic flea. The ink was then cast as a thin film (ca. 80 μm when wet and ca. 24 μm dry) onto 50 μm PET film using a K-bar 7 coater;[22] the dry film is colourless in air (due to the presence of 0.041% CO_2). MeOH is used here, and in most reported CO_2 indicator preparations,[12-15] as one of the solvents, since the quaternary base is usually sold as a methanol-based solution. Given its low boiling point, and the fact that the indicator is well dried before use, it is unlikely the MeOH, or the other solvents used in the preparation of the ink film, present any significant health risk. However, it is worth mentioning the OCP indicator also works well when covered with a thin layer of Sellotape which, if required, would then ensure the ink film does not make direct contact with the packaged product.

Methods

All UV-vis absorbance measurements were made using an Agilent Technologies CARY 60 UV-vis spectrophotometer. All digital photographs were taken using a Cannon 600D digital camera and all digital images were processed for their red, green and blue colour space values (*i.e.* RGB values) using the freely available photo-processing software, Image J.[23] In one part of the work, a DVP vacuum technology, model: LC.4, vacuum pump was used to evacuate the spectrophotometric cell containing the indicator. In another part, a vacuum chamber, a model: DP1.5 chamber supplied by Applied Vacuum Engineering, was used to monitor simultaneously; (i) the ambient vacuum pressure (measured using a digital vacuum meter, a Keller-Druk Digital manometer, model: LEO Record, placed inside the chamber) and (ii) the colour of the indicator (through digital photography). All food was packaged using a commercial food vacuum packager made by Orved, model: Multiple 315. Unless stated otherwise, all work was carried out at 22 ^oC.

3. Result and Discussion

Qualitative analysis

As noted above the super-sensitive CO_2 ink containing OCP, the 'vacuum indicator', was colourless in air, but when placed in a 1 cm spectrophotometer cell and evacuated using a vacuum pump, the film quickly developed a striking violet colour, as illustrated by the digital images taken of the indicator in figure 1 (a). The vacuum in the cell (< 0.002 atm) was then slowly released, over 2 h, allowing ambient air to enter into the cell, and bring with it sufficient CO_2 so as to bleach the indicator, *via* reaction (1), given the deprotonated and protonated forms of OCP are violet and colourless, respectively. Both the photographed colour of the indicator and its UV-vis absorption spectrum were monitored during this bleaching process and the results of this work are illustrated in figure 1(a) and (b), respectively. These results show that the vacuum indicator can be used at least as a *qualitative* indicator of sub-ambient, *i.e.* low, air pressures.

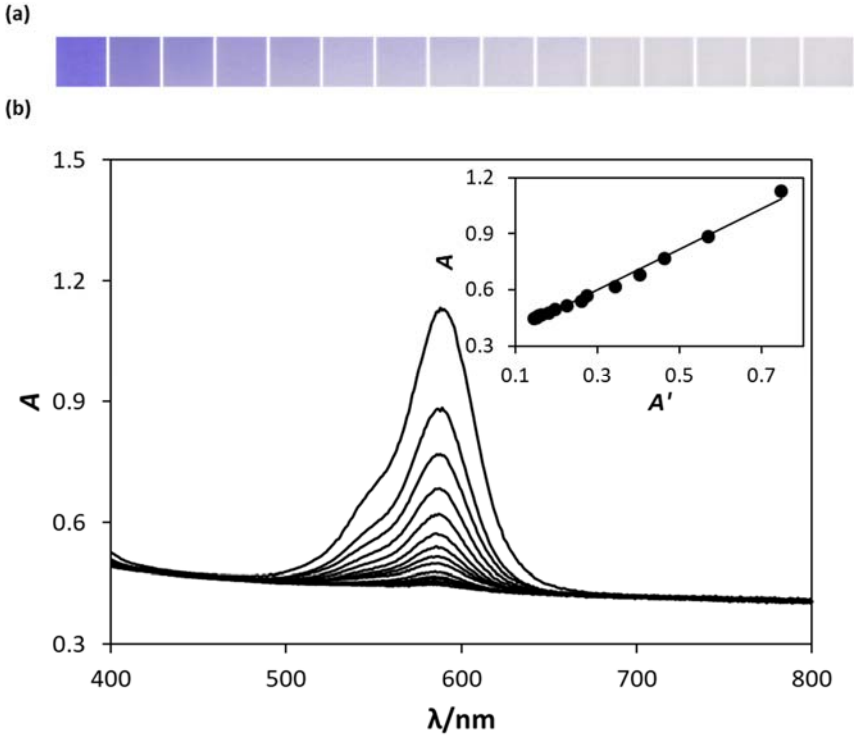

Fig. 1: (a) Digital photographs of the OCP vacuum indicator in an evacuated cuvette as, from left to right, air is slowly – over 2 h - allowed in and (b) simultaneous recording of the UV-vis absorption spectrum of the vacuum indicator (from top to bottom). The insert diagram is a plot of the real absorbance, *A*, vs the apparent absorbance, *A'*, *vide infra*.

Digital and spectrophotometric analysis

Recent work by this group and others has shown that[24-26] for simple colour-changing systems, such as here, digital photography coupled with colour analysis, using red, green and blue colour space values (RGB) obtain from freely available software such as *Image J*,[23] can be used to generate apparent absorbance values, A', which are directly proportional to their real absorbance value, A, counterparts. Apparent absorbance values are calculated using either the red, green or blue component values (ranging from 0-255) of the digital image of the indicator, *i.e. RGB(red)*, *RGB(green)* and *RGB(blue)*. In the case of the OCP vacuum indicator, the *RGB(red)* component varied most significantly with P and so its values, derived from the digital images illustrated in figure 1(a), were used to calculate the apparent absorbance values, A', for the vacuum indicator, using the expression:

$$A' = \log\{255/RGB(red)\} \qquad (3)$$

The direct relationship between the real, A, and apparent absorbance, A', values for the vacuum indicator is demonstrated by the good fit to a straight line of the data in the insert plot in figure 1(b). In this plot the values of A were derived from the UV-vis absorption spectral data in the main diagram of figure 1(b) and the values of A', the apparent absorbance values, were derived from colour analysis, and eqn (3), of the associated digital photographs in figure 1(a). The straight line nature of this plot suggests that simple digital colour photography, coupled with colour analysis, can be used to probe the vacuum pressure response characteristics of the vacuum indicator, instead of the much more expensive and cumbersome method of UV-vis spectroscopy. Note that the digital image colour analysis reported here was carried out here using the free software Image J[23] but can also be achieved using a mobile phone and colour analysis App of which there are many, as they are often used as an indoor decorating tool to help identify and reproduce a particular colour, as in of paint for example.[27-29] This ease of analysis is an important feature if the vacuum indicator is to be used routinely as a *quantitative* method for measuring vacuum pressure, as well as (by eye) a *qualitative* method to identify if the vacuum in the pack is still present or not.

Quantitative analysis

The absorbance at the λ_{max} of D^-, *i.e.* 589 nm, of the OCP dye in the vacuum indicator film, *A*, is a measure of the concentration of $[D^-]$ and can be used, *via* eqns (1) and (2), to calculate a value for, *R*, which is related directly to $\%CO_2$, since:

$$R = (A_0 - A)/(A - A_\infty) = [HD]/[D^-] = \alpha.\%CO_2 \qquad (4)$$

where, A_0 is the value of absorbance due to the pH-indicating dye at $\lambda_{max}(D^-)$ when $\%CO_2 = 0$ (*i.e.* when all the dye is in its deprotonated form) and A_∞ is the absorbance of the film when all of D is in its protonated (colourless) form, *i.e.* as HD, *i.e.* when $\%CO_2 = \infty$; the latter absorbance is assumed here to be that measured when the $\%CO_2$ is that of air, *i.e.* = 0.041 % and the film is colourless. In this work the source of CO_2 is ambient air, and so it follows that $\%CO_2$ is proportional to the air pressure in the cell (or package), *P*, and so eqn (4) can be rewritten:

$$R = (A_0 - A)/(A - A_\infty) = \alpha'.P \qquad (5)$$

where α' is measure of the sensitivity of the indicator (units: atm^{-1}). Note also that, since the real absorbance, *A*, (from spectrophotometric measurements) is proportional to the apparent absorbance, *A'*, (from photographic measurements), eqn (5) can be re-written as:

$$R = (A'_0 - A')/(A' - A'_\infty) = \alpha'.P \qquad (6)$$

The above expression suggests that digital photography, coupled with colour analysis, can be used to provide a measure of the ambient vacuum air pressure, *P*. In order to test this idea, the vacuum pressure indicator was placed in a vacuum chamber which had a clear glass top so the indicator could be photographed, along with a digital manometer, and evacuated down to ca. 0.002 atm at which point the originally colourless vacuum indicator was rendered a bright violet colour. Air was then very slowly allowed into the system and the vacuum pressure (monitored using the digital manometer) and indicator colour (monitored by digital photography) recorded as the air pressure increased so that the vacuum indicator returned to its colourless (protonated) form. As noted earlier, all this work was carried out at 22 ^oC and the digital images of this experiment collected over this time are illustrated in figure 2(a). RGB colour analysis of each of these digital images coupled with eqn (3), allowed the calculation of the apparent absorbance value, *A'*, of the vacuum indicator at each pressure, P. This data was used to construct the straight line plot of R, calculated using eqn (6), vs P, illustrated in figure 2(b), (solid black dots) for the VP indicator at 22 °C, from the gradient of which a value for α', of 16.1 ± 0.9 atm^{-1} was calculated. A measure of the pressure sensitivity of the vacuum indicator is the value of $1/\alpha'$, since this corresponds to the pressure at which the indicator will have lost half its initial violet colour, *i.e.* the point when R = 1 and [D^-] = [HD] and for the vacuum indicator $1/\alpha'$ = 0.062 atm at 22 °C. Given a typical vacuum packaged food product will be at ca. 0.04 atm,[1] this value of α', and so the vacuum indicator itself, appear well-suited for monitoring the pressure inside a vacuum-packed product, so that the indicator is able to reveal any subsequent loss of vacuum pressure, due to a pin-hole leak say, after the initial vacuum packaging process. The straight line plot illustrated in figure 2(b) suggests that, provided the temperature is known, a photographic image of the vacuum indicator inside a vacuum packaged product can be used to provide a measure of the ambient vacuum pressure, P, inside the pack, *i.e.* the vacuum indicator can be used for *quantitative* analysis of ambient vacuum air pressure in a vacuum-packaged product.

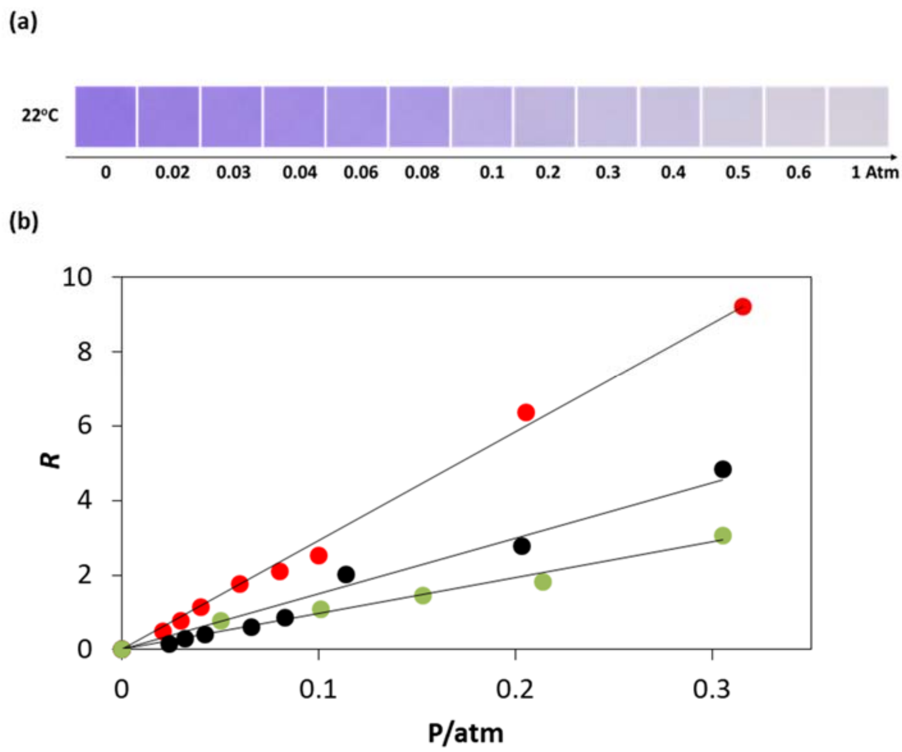

Fig. 2: (a) Digital photographs of the OCP vacuum indicator in an evacuated chamber with pressure gauge as, from left to right air is slowly –allowed in at 22 °C; and (b) plot of R vs P for the same system, where R values were calculated using eqn (6) and apparent absorbance values derived from colour analysis of the images of the VP indicator at 17 (red dots), 22 (black dots) and 28 °C.

Temperature Sensitivity

The same experiments reported above, for probing the *quantitative* analysis properties of the vacuum indicator at 22 ^{o}C, were also carried out at 28 and 17 ^{o}C and the subsequent plots of *R* vs *P* are also illustrated in figure 2(b). From the gradients of these straight line plots, values of α' of 9.1 $\pm$ 0.8 atm^{-1} and 29.9 $\pm$ 0.7 atm^{-1} were calculated for 28 and 17 ^{o}C, respectively. Since α' is an equilibrium constant, it is expected, from the van't Hoff equation, that a plot of ln(α') vs 1/T would yield a straight line with a gradient = -ΔH/R, where ΔH is the enthalpy change associated with reaction (1), and with OCP as the pH indicating dye. Not surprisingly, therefore, a plot of ln(α') vs 1/T, using the three values of α' reported above, yielded a good straight line, with a gradient that revealed a value for ΔH of 78 $\pm$ 5 kJ mol^{-1} which is in line with those reported for other CO_2-sensitive colourimetric indicators (*i.e.* 32-77 kJ mol^{1}).[9]

Response and recovery

The response and recovery characteristics of the vacuum indicator were briefly explored by placing the indicator in a vacuum chamber and exposing it to a cycle of a relatively low (< 0.001 atm) air pressure (rendering it violet coloured) following by ambient air pressure, 1 atm, (rendering it colourless), whilst at the same time monitoring the colour changes *via* digital photography. The apparent absorbance values, *A'*, associated with these colour changes were determined from the digital images, and the results of this study are illustrated in figure 3 and reveal 90% response and recovery times for the vacuum indicator, *i.e.* $t_{90}\uparrow$ (colourless to violet; air to vacuum) and $t_{90}\downarrow$ (violet to colourless; vacuum to air), respectively, of 16.2 and 2.7 min, respectively. These timescales appear reasonable given the suggested application of the indicator, namely as a package integrity indicator for vacuum packaged food.

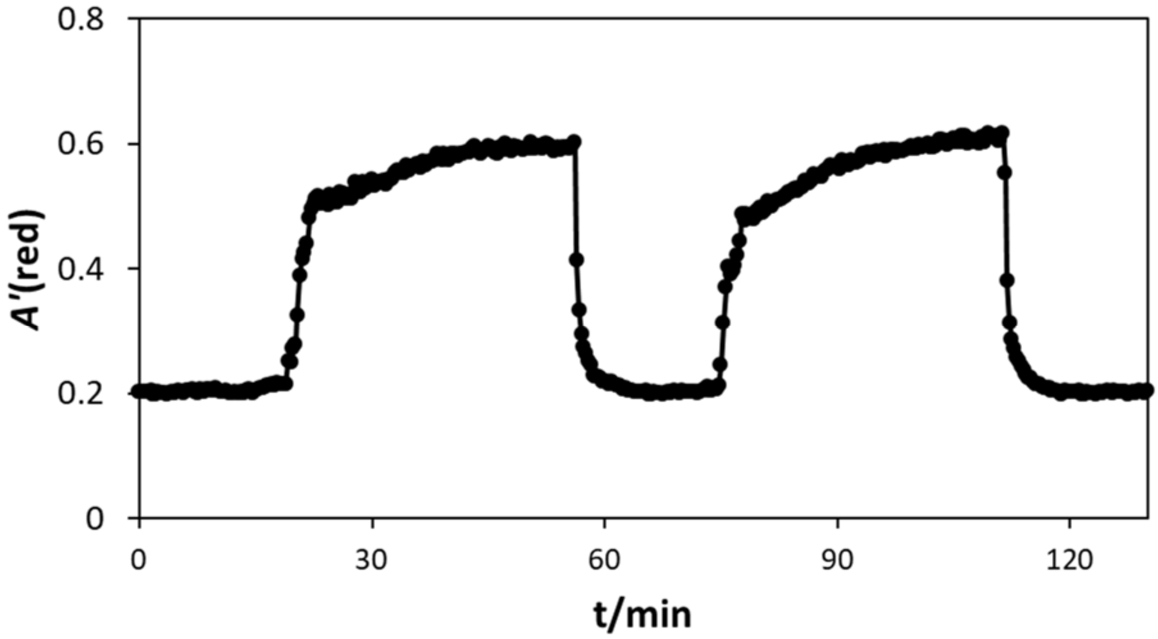

Fig. 3: Variation in the apparent absorbance (*A'*) of the OCP vacuum indicator as a function of time as it was exposed to a cycle of low (< 0.001 atm) and high (1 atm) air pressure, from which $t_{90}\uparrow$ and $t_{90}\downarrow$ values of 16.2 and 2.7 min, respectively, were calculated.

Testing with a vacuum packaged food product

Finally, the vacuum indicator was stuck to piece of white card which was then placed inside a store-bought bag of vacuum-packaged risotto rice.[30] The product, plus indicator, was then evacuated using a commercial food vacuum packager and, as expected, the indicator rapidly turned bright violet and remained at that colour until opened, when it was immediately rendered colourless. From colour analysis of the digital photograph of the OCP indicator in the vacuum-packed rice, and eqn (3), an apparent absorbance value, *A'*, of 0.38 was calculated, which in turn was used to calculate a value of *R* of 1.37 which, using the calibration graph illustrated in figure 2, indicates that the vacuum pressure inside the pack was 0.085 ± 0.005 atm. This estimated pressure compared very well with that measured by the digital vacuum meter, 0.084 atm, when the latter was vacuumed packed under the same conditions. Photographs of the package plus indicator before and after opening, and the vacuum packed digital vacuum meter are shown in figure 4.

Fig. 4: Digital photograph of the vacuum packed risotto rice with OCP indicator, before (left) and after opening (middle), and vacuum packed digital vacuum meter.

Conclusions

The above work shows that it is possible to create a vacuum pressure indicator for both *quantitative* (as in figure 3) and *qualitative* applications, using a very sensitive CO_2 indicator, based on the pH indicator dye, OCP. Such an indicator may well find an application in vacuum packaging, for which at present there does not exist a simple, inexpensive method for 100% quality control. Although the example illustrated in figure 4 is for vacuum packaged food, other vacuum packaged goods, such as pharmaceuticals, medical instruments and electronic components, may also benefit from this technology, such as pressure sensitive paints. This is the first reported colourimetric plastic film vacuum indicator and many more are likely to follow given that they can be readily tuned to respond to different air pressures by using different pH-indicating dyes, with different pK_a values, and different base concentrations.[11]

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