Journal Pre-proofs

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Please cite this article as: Veríssimo, M.I.S., Gamelas, J.A.F., Fernandes, A.J.S., Evtuguin, D.V., Teresa S.R. Gomes, M., A new formaldehyde optical sensor: detecting milk adulteration, *Food Chemistry* (2020), doi: https://doi.org/10.1016/j.foodchem.2020.126461

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A new formaldehyde optical sensor: detecting milk adulteration

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Abstract

A sensor consisting of an optic fibre with the exposed tip coated with a the polyoxometalate salt $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$, specially designed to be insoluble in water, which UV-Vis spectrum changed in contact with formaldehyde, is presented.

The sensor limit of detection for formaldehyde was 0.2 mg L^{-1}, and the limit of quantification was 0.6 mg L^{-1}, which were close to the conventional spectrophotometric method values of 0.2 mg L^{-1} and 0.5 mg L^{-1}, respectively, and lower than the tolerable limit for ingested food. The sensor was tested for formaldehyde quantification in milk, as its deliberate addition is a matter of concern. The results obtained analysing formaldehyde in milk samples by the optical sensor and by the conventional method were not statistically different ($\alpha=0.05$).

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1. Introduction

Milk is an excellent source of protein, fat, carbohydrate, minerals and vitamins, not just for humans, but also for other mammals. Therefore, measures to deliver a safe product with the desired nutritional and organoleptic characteristics dominate the industry.

Milk adulteration is still a matter of concern affecting mainly underdeveloped countries, but requiring surveillance worldwide (Chakraborty & Biswas, 2018; Handford, Campbell, & Elliott, 2016; Moore, Spink, & Lipp, 2012). Strict legislation must be accompanied with the availability of sensitive, low cost and easy to use analytical instruments and methods.

Milk adulterants are typically related with: i) adulteration of nitrogen content, ii) adulteration of the fat content of milk and addition of detergents, iii) adulteration with water and other substances and iv) adulteration to increase the product shelf life (Nascimento, Santos, Pereira-Filho, & Rocha, 2017). Formaldehyde belongs to the last group and it is quite effective in decreasing microbial growth and thus increasing the product shelf life. Formaldehyde has been added not only to milk for human consumption but also to skimmed milk used for animal feeding (Herschdoerfer, 1968).

Although formaldehyde is a natural metabolic product of the human body, high-dose exposure increases the risk of acute poisoning, while prolonged exposure can lead to chronic toxicity and even to cancer. The International Agency for Research on Cancer (IARC) has classified formaldehyde as carcinogenic to humans (group 1) (IARC Working Group, 2018). The United States Environmental Protection Agency (US EPA) provides a reference dose for chronic oral

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exposure (RfD) of 0.2 mg per kg of body weight per day (U.S. Environmental Protection Agency (EPA), 1989). The World Health Organization - International Programme on Chemical Safety (WHO IPCS) has established a tolerable concentration (TC) of 2.6 mg L^{-1} in ingested products, based on animal experiments (World Health Organization, 2017).

Liquid whey is a sub-product of cheese manufacture, in quantities much higher than those prone to be incorporated into animal and human foods, and finds value on freshly use to feed livestock. Unfortunately, storage before transportation is often necessary. Keeping whey fresh could be done by cooling, pasteurising, or by adding preservatives (El-Tanboly, El-Hofi, & Khorshid, 2017). Formaldehyde is one of the most economical and effective preservatives to avoid curdling (Buckley, Fisher, & MacKay, 1988; El-Tanboly et al., 2017). Therefore, formaldehyde use in animal feeding has been going on after its prohibition in food for humans. Recently, a requested authorisation for continuing to use it in animal feeding was refused and the technological formaldehyde pre-mixtures for skimmed milk to feed less than six months swines were banned from the market at 28th May 2018. EU legislation forced the withdraw of skimmed milk samples containing the formaldehyde additive till 28th August 2018 (European Commission, 2018). Therefore, effective rapid and affordable methods for formaldehyde detection and quantification in food, both for human and animal consumption are urgently required.

Gas chromatography-mass spectrometry (GC-MS) after derivatisation of formaldehyde to pentafluorophenylhydrazone (Jeong et al., 2015; Sugaya, Nakagawa, Sakurai, Morita, & Onodera, 2001), but most frequently high-performance liquid chromatography (HPLC) with UV detection, after derivatisation with 2,4-dinitrophenylhydrazine (DNPH), have been mostly used. Other techniques usually employed are spectrophotometry and fluorometry, most often based on Hantzsch reaction (ATSDR, 1992; Hossain, Islam, & Bhadra, 2016; Li, Sritharathikhun, & Motomizu, 2007; Nash, 1953; Wahed, Razzaq, Dharmapuri, & Corrales, 2016).

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The acetylacetone method, as it is often designated the colourimetric method based on the Hantzsch reaction of formaldehyde with acetylacetone in the presence of ammonia to form a yellow product of 3,5-diacetyl-1,4-dihydrolutidine (DDL) (Li et al., 2007; Nash, 1953), has been considered a standard method of analysis by the Agency for Toxic Substances and Disease Registry (ATSDR, 1992). This method is extremely selective and sensitive and the formation of the complex takes place in neutral medium (Isai & Vas'kovskii, 1973). It has been widely used to determine formaldehyde in food (Jaman, Hoque, Chakraborty, Hoq, & Seal, 2015; Noordiana, Fatimah, & Farhana, 2011; Nowshad, Islam, & Khan, 2018), despite including a heating step and being relatively slow. The major drawback of all the mentioned methods relies on the fact that they are all analytical laboratory methods, not suitable to be used in the field.

Colourimetric methods based on paper impregnated with the necessary reagents for the Hantzsch reaction (Guzman, Tayo, Liu, Wang, & Fu, 2018), or on biodegradable starch colourimetric film (Wongniramaikul, Limsakul, & Choodum, 2018) and with colour detection by a CMOS camera or by the digital camera of an iPhone, are examples of endeavour to simplify the instrumentation. Although one of the methodologies employs microfluidics in an attempt to reduce sample/reagent volume and increasing operational simplicity and throughput, heating continues to be an indispensable step. The lack of fit-for-purpose equipment leads to the wrong use of portable commercial instruments, such as the formaldehyde Meter Z-300 by Environmental Sensors Co, USA. Although it has been designed to monitor formaldehyde in air, inspectors and mobile courts in Bangladesh are using the device in food analysis, with doubtful results (Hossain et al., 2016).

This work presents a new approach for formaldehyde detection, using an optical fibre pigtail coated with a sensitive layer. Although extrinsic fibre optic devices have already been published for formaldehyde detection in milk (Saracoglu & Hayber, 2016), formaldehyde detection in those devices was based on refractive index changes, which are far from being selective. In this work, the presence of the sensitive membrane at the exposed end of the optical fibre turns the optical

fibre into a selective intrinsic chemical sensor, without losing its appealing low weight and non-electric characteristics.

The correct choice of the sensitive membrane, based on a polyoxometalate compound, was essential. Polyoxometalates (POMs) are a class of metal-oxygen cluster anions (comprising Mo, W and V as main primary heteroatoms), well-known for their structural and electronic properties, and with a wide range of potential applications, namely in oxidative catalysis (Gaspar, Gamelas, Evtuguin, & Pascoal Neto, 2007). In a previous work, the $H_5[PMo_{10}V_2O_{40}]$ heteropoly acid was used as a sensitive layer of a piezoelectric quartz crystal for 5-hydroxymethylfurfural determination in honey (Veríssimo, Gamelas, Evtuguin, & Gomes, 2017). This POM compound allows detection of reducing gases after careful drying of all the contacting gases. However, although this compound showed ability to interact with aldehydes while changing its colour from orange to green (vanadium reduction from V^V to V^{IV}) and to easily recover its original form, becoming orange again quite easily, its solubility in water prevents its use in aqueous solution. The $H_5[PMo_{10}V_2O_{40}]$ heteropoly acid, as well as cyanometalate-functionalized POMs, have recently been incorporated in formaldehyde metal oxide semiconductor gas sensors, but as enhancer for selective formaldehyde sensing. Photoconductive semiconductor sensors modified with POM have been used for the formaldehyde detection in gaseous media, where the presence of POM was claimed to retard the electron-hole recombination process and thus to increase the photocurrent response. In the presence of the analyte gas, due to an adsorption process/analyte oxidation, the photocurrent was enhanced, increasing with the analyte concentration (P. Wang et al., 2018; T. Wang et al., 2017).

Another POM compound, the polyoxotungstate salt, $[(C_4H_9)_4N]_4[PW_{11}Mn^{III}(H_2O)O_{39}]$, with tetrabutylammonium (TBA) as the counter cation, which renders the compound insoluble in water, has been considered later. Unfortunately, this POM did not experience a colour change while in contact with reducing compounds, and therefore it would not be possible to use it for optical

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sensing. Other transducer mechanism needed to be used, and it was possible to use it as a sensitive layer over a piezoelectric quartz crystal to determine acetaldehyde in ciders (Veríssimo, Gamelas, Simões, Evtuguin, & Gomes, 2018). Therefore, the approach of using POM in optical sensors has never been presented before.

In this work the $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ was synthesised and characterized, the sensitive membrane was prepared, and after coating the optical fibre, POM distribution within the sensor membrane was visualized. Milk samples were analysed by the optical sensor and by the well-established spectrophotometric acetylacetone method. Results were compared in order to evaluate the reliability of the new device.

2. Materials and methods

2.1 Reagents and standards preparation

Tetra-*n*-butylammonium bromide used in the preparation of the $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ compound, and dimethyl sulfoxide-d6 (99.9 atm% D) used for the acquisition of the corresponding ^{31}P NMR spectrum were purchased from Sigma-Aldrich (Madrid, Spain).

POM was incorporated into a membrane formed with polyvinyl chloride (PVC) and *o*-nitrophenyl octyl ether (NPOE) from Fluka (Steinheim, Germany) in tetrahydrofuran (THF) from Merck (Darmstadt, Germany).

Formaldehyde (37%, w/v) from Merck was used to prepare a stock solution (approximate concentration 4000 mg L^{-1}), which was standardized (National Institute of Occupational Safety and Health, 2003) using sodium sulfite and sodium hydroxide from Panreac (Barcelona, Spain) and sulfuric acid and potassium biphthalate from Merck.

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A diluted formaldehyde solution (approximately 400 mg L^{-1}) was prepared daily from the standardized stock solution and was used to prepare aqueous formaldehyde standard solutions (until 8.5 mg L^{-1}), by appropriate dilution with Milli-Q water, and for spiking the samples in the standard addition calibration method. Ultrapure water was produced by a Milli-Q system from Millipore (Mississauga, Canada).

Ammonium acetate, acetic acid and acetylacetone supplied by Merck and ammonium hydroxide from Fluka were used in the acetylacetone spectrophotometric method.

Trichloroacetic acid from Panreac was used to precipitate proteins from the milk samples.

2.2 Samples and samples' preparation

Commercial milks from several brands were bought in local supermarkets. Powder milks for human consumption were kindly supplied by the local industry. Milks from powder were prepared according to the manufacturer's specifications.

Prior to the analysis, all milk samples were clarified, by precipitating the proteins with trichloroacetic acid (TCA) (Nascimento et al., 2017). Aliquots of 6.00 mL of milk and 3 mL of TCA (20%) were centrifuged in 15 mL Falcon tubes at 4000 rpm for 5 minutes. A centrifuge Mixtasel Mod. 540 from Selecta (Barcelona, Spain) was used. The supernatant was then filtered through Whatman nº1 filter paper (Maidstone, England). The pH was adjusted to 6.0-6.5 with NH$_4$OH, using a pH & Ion-meter GLP 22$^+$ from Crison (Barcelona, Spain), transferred to a 50.00 mL volumetric flask, and final volume adjusted with Milli-Q.

2.3 POM synthesis and characterization

H$_5$[PMo$_{10}$V$_2$O$_{40}$]·11H$_2$O was prepared following an already described procedure (Tsigdinos & Hallada, 1968) and the synthesis of the [(C$_4$H$_9$)$_4$N]$_4$H[PMo$_{10}$V$_2$O$_{40}$] was made by metathesis of the corresponding heteropoly acid, as follows: 0.40 g of tetra-*n*-butylammonium bromide was

added to a solution of 0.40 g of $H_5[PMo_{10}V_2O_{40}]\cdot11H_2O$ dissolved in 10 mL of water; an orange solid was formed immediately; the suspension was stirred for one hour, after which the solid was filtered off, washed with four 5 mL portions of water and dried in a vacuum desiccator. The $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ compound (0.5 g) was further characterized by thermogravimetric analysis, ^{31}P nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy. Thermogravimetric analysis was done on a TGA-50 Shimadzu thermobalance (Kyoto, Japan) under air atmosphere in the 25-700 ºC range at a heating rate of 10 ºC min^{-1}. ^{31}P NMR spectrum was recorded on a Bruker Avance III 400-MHz NMR spectrometer (Massachusetts, USA). For the ^{31}P NMR analysis, the sample was dissolved at room temperature in deuterated dimethyl sulfoxide and put inside a 5-mm NMR tube for the spectrum acquisition. Signals were referenced to phosphoric acid (85%). FTIR spectrum of the sample in potassium bromide pellet was obtained in a Mattson 7000 spectrometer. Spectrum was recorded in the 300-4000 cm^{-1} range with a resolution of 4.0 cm^{-1} and a number of scans of 256.

2.4 Coating of the optical fibre and membrane characterization

The sensitive membrane was prepared by mixing PVC (33%), NPOE (66%) and $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ (1%) in 1 mL of THF.

The optical fibre, from Sarspec (Vila Nova de Gaia, Portugal) had a pure fused silica core of 600 μm. To prepare the fibre to be coated, around 1 cm of the external buffer was cut with a blade and removed, exposing the cladding. Then, a Micro-strip from Micro Electronics Inc. (Massachusetts, USA) was used to remove the cladding of the fibre, exposing around 2 to 3 mm of the fibre core. Finally, the core was gently cleaned with ethanol before coating. The exposed core of the fibre was then dipped into the membrane solution, removed, and left to dry for at least 3 hours, but preferably overnight.

To corroborate the presence of the $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ on the surface of the exposed core of the optical fibre, and to observe its distribution within the membrane, two small pieces of the core of the optical fibre (1 cm long, 1000 μm thick) coated with a PVC/NPOE membrane and another with the PVC/NPOE/$[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ membrane were analysed in a high resolution scanning electron microscope (SEM) Tescan Vega3 XMU with an accelerating voltage of 25 keV, coupled with a spectral analysis system of X-ray dispersive energy (EDS) Bruker Quantax 400. The optic fibre samples were fixed in a cylindrical sample holder with conductive carbon.

2.5 Optical sensor set-up

The experimental set-up employed is shown in Fig. 1. The sensing fibre of 600 μm (core), from Sarspec, was connected by one end to the UV-Vis light source DH-2000 from Ocean Optics (Florida, USA) with a SMA-905 connector, and by the other end (the coated one), to a PEEK home-made cell, possessing a 1.5 mL reservoir with a filling entrance at the top, and an exhaust aperture at the bottom. The light leaving the coated optical fibre was focused in a convex flat lens 1'', with a focal distance of 30 mm, from Sarspec, and sent to the spectrophotometer ScanSpec UV/Vis from ScanSci (Vila Nova de Gaia, Portugal), through another pure fused silica fibre of 600 μm (core), also from Sarspec. The spectrophotometer was connected to a PC with a USB cable. The acquisition software was Spectrascan from Scansci.
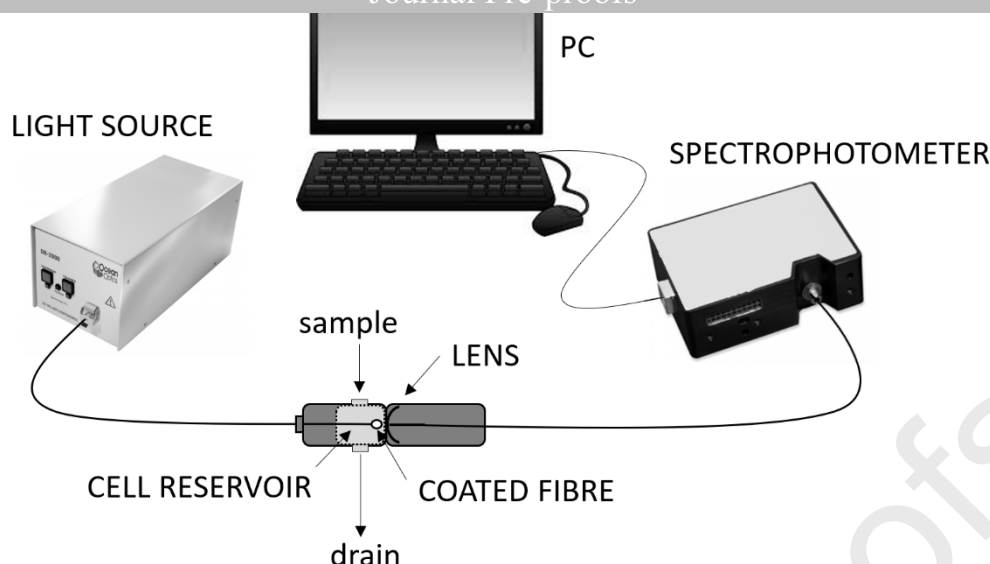
Fig. 1. The experimental setup used with the optical sensor.

Experiments were also performed using a less intense UV-Vis light, ScanSource from Scansci replacing the sensing 600 µm core optical fibre with a 1000 µm pure silica core, also from Sarspec.

2.6 Procedure for formaldehyde analysis

2.6.1 The acetylacetone spectrophotometry

The acetylacetone method (Jaman et al., 2015; Noordiana et al., 2011; Nowshad et al., 2018), which uses Nash reagent, was used as an alternative to determine formaldehyde in solution. Briefly, Nash reagent was prepared by dissolving 15.0 g of ammonium acetate, 0.3 mL of acetylacetone, and 0.2 mL of acetic acid in 100 mL of Milli-Q water. Ammonium hydroxide was used to adjust pH to 6.0-6.5. Nash reagent is light sensitive and was kept in a dark glass bottle.

3 mL of Nash reagent were added to 5.00 mL of each of the formaldehyde standards (seven standards from LOQ until 3.04 mg L^{-1}) or to 5.00 mL of the clarified milk samples, followed by heating in a water bath for 15 min at 60 ºC. After cooling the absorbance at 415 nm of the standards and samples were immediately measured, using a double beam T90+ UV/Vis spectrophotometer

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from PG Instruments Ltd (Leicestershire, United Kingdom). The absorbance of each standard/sample was read in triplicate.

2.6.2 The optical fibre sensor

Analyses were performed using the setup reported in Fig. 1. Initially, light sources (deuterium and halogen lamps) were left to stabilize for 30 minutes. Spectrophotometer calibration was performed with the uncoated optical fibre inside the home-made cell: Dark was used to set the zero transmittance and the Reference (100% transmittance) was set with the uncoated optical fibre at light saturation. The optical fibre was afterwards removed from the home-made cell, coated with the POM membrane, allowed to dry, and introduced back into the cell. The absorbance spectrum of the coated optical fibre was measured while the cell was empty. Acquisition parameters were set at: Integration time 400 ms, Average 30 and Boxcar 5, and were maintained for all further measurements, which included spectra obtained after filling the cell with water, standards or samples.

With the bottom hole closed, the sample to be analysed was poured through the top hole of the cell with a Pasteur pipette, and the chamber top was closed with a screw thread. Care was taken to avoid air bubbles. The UV-Vis spectrum (in absorbance units) was saved after stabilization. After the measurement, the chamber was opened, unscrewing the top and bottom screws, and the sample was evacuated by gravity. The cell reservoir was washed with Milli-Q water between analysis.

3. Results and discussion

3.1. $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ characterization

A compound was idealized, which could experience a colour change while in contact with reducing compounds, like aldehydes, through a mechanism which does not involve significant energy, so that reversibility would be easily attained, but insoluble in water. The decamolybdodivanadophosphate salt, $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$, with TBA as counter-cation meets the water insolubility requirements as well as the optical activity, turning it an excellent candidate as the sensitive film of an optical sensor to be used in aqueous solutions.

The $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ compound was characterized by thermogravimetry, ^{31}P NMR spectroscopy and FTIR spectroscopy. The thermogravimetric plot (Supplementary Material, Fig. S1) showed a weight loss from ca. 240 ºC to 490 ºC of 37.37%, in agreement with the formulae $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ (theoretical value: 37.40%). The water content of the compound was negligible ($\leq$0.01%).

The ^{31}P NMR spectrum of the compound in dimethyl sulfoxide (Supplementary Material, Fig. S2) showed several peaks within the -2.5 ppm to -3.5 ppm range, namely a signal at -3.48 ppm, attributed to $PMo_{11}V$, signals at -3.27, -3.15, -3.01, -2.97 and -2.88 ppm, due to several isomers of the $PMo_{10}V_2$ anion. Less intense signals at -2.73, -2.62 and -2.58 ppm were also observed, most probably due to isomers of PMo_9V_3 species. The signals of $PMo_{10}V_2$ accounted for ca. 65% of the spectrum, while that of $PMo_{11}V$ corresponded to 27% and those from PMo_9V_3 to ca. 8%. As common for the related heteropoly acid (Gamelas, Evtyugina, Portugal, & Evtuguin, 2012; Pettersson, Andersson, Grate, & Selling, 1994), the POM compound also showed several species, and, therefore, the formulae "$[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$" may be seen as only an approximate one.

The FTIR spectrum of $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ (Supplementary Material, Fig. S3) showed bands at 2960, 2935, 2873, 1481 and 1380 cm^{-1} due to the C-H stretching and C-H bending vibrations in the tetraalkylammonium groups. Bands showing the presence of the Keggin-type

polyoxometalate were observed at 1074 and 1054 cm⁻¹ (P-O), 944 cm⁻¹ (W=O), 873 and 798 cm⁻¹ (W-O-W) (Nomiya, Yagishita, Nemoto, & Kamataki, 1997).

3.2 PVC/NPOE/[(C₄H₉)₄N]₄H[PMo₁₀V₂O₄₀] membrane characterization

Two small pieces of 1 cm (length) fused silica (1000 μm core) coated with PVC/NPOE membrane, one with and the other without $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ were analysed by SEM/EDS. Although surface patterns of both surfaces were very similar, it was possible to find some agglomerates of white colour on the one coated with the POM doped membrane which is shown in Fig. 2a. The observed agglomerates indicate that the salt was not uniformly dispersed in the polymer matrix. Both SEM/EDS images of the fibre coated with PVC/NPOE membrane, undoped and doped with POM, can be found in Supplementary Material, Fig. S4 and Fig. S5, respectively, where the strongest signals belong to Cl (2.6 keV) and C (0.25 keV) from the PVC membrane.
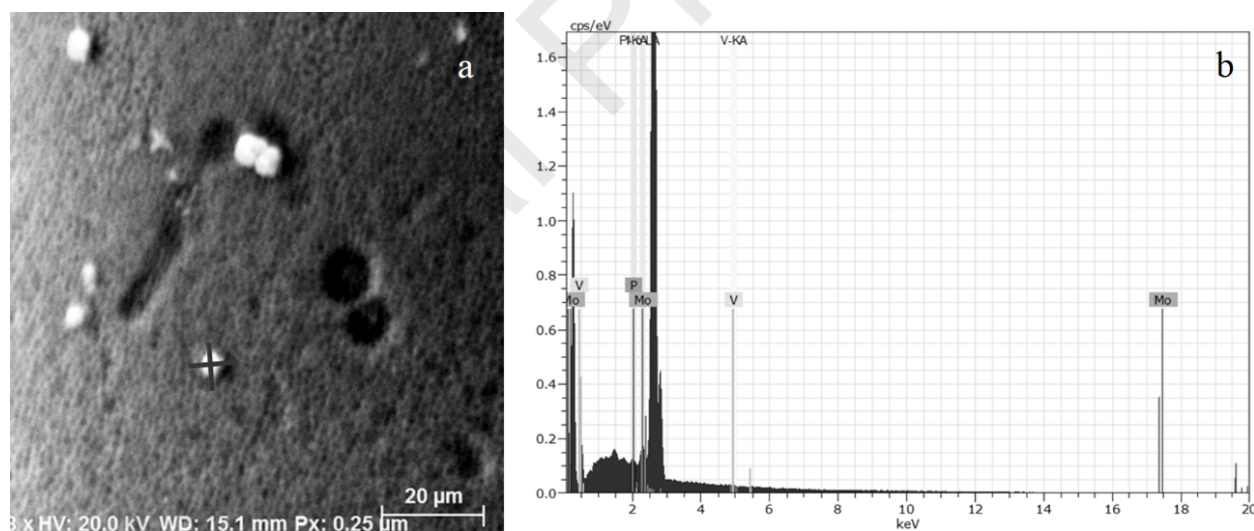

Fig. 2. a) SEM image of a PVC/NPOE membrane doped with $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ (1%) and b) EDS analysis of the salt agglomerate on the membrane surface (marked with a cross on Fig.2a).

Fig. 2b shows the EDS spectrum of the selected point (cross) in Fig. 2a. The EDS spectrum confirms the Mo and P presence, both from POM, that were absent in the EDS spectrum of the undoped membrane (Fig S4, Supplementary Material).

3.3. Sensitive membrane UV-Vis spectrum and wavelength selection for formaldehyde analysis

Fig. 3 shows the UV-Vis spectrum of the dried coated fibre inside the empty cell. Two well-defined absorption bands at 261 and 329 nm can be observed, due to the presence of the $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ at the tip of the fibre (O→Mo/V charge transfer bands) (Pope, 1983). In the present configuration, with the described equipment and acquisition parameters, no other bands were detected.
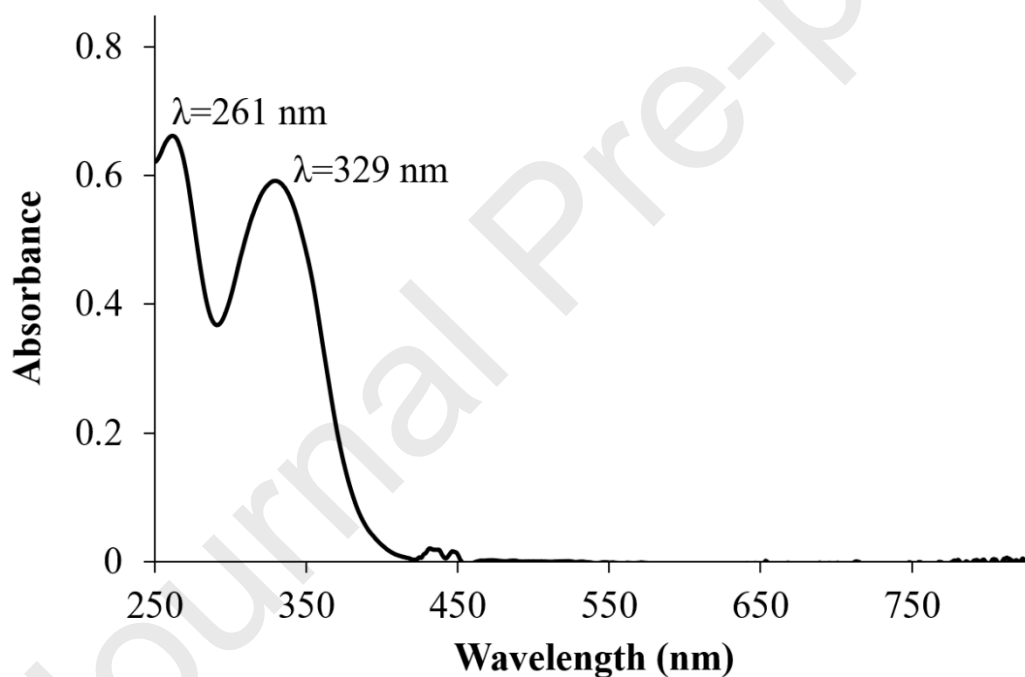
Fig. 3. UV-Vis spectrum of the PVC/NPOE/$[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ membrane.

Fig. 4 shows the UV-Vis spectra obtained after filling the cell with water and formaldehyde solutions.
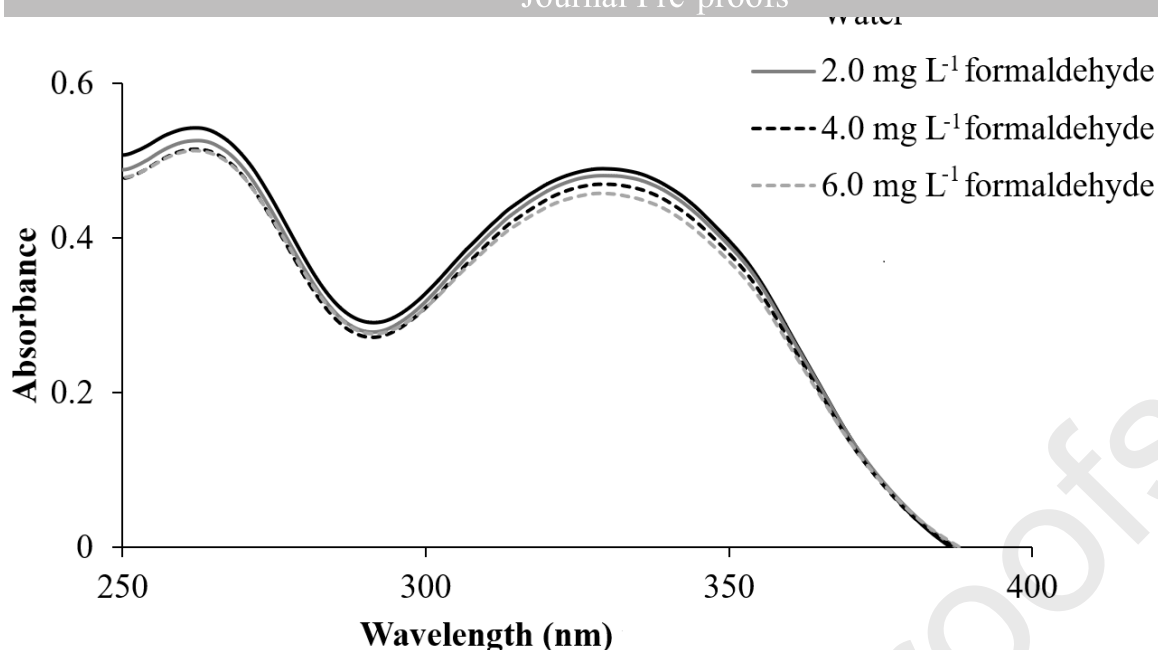
Fig. 4. UV-Vis absorbance spectra of the optical sensor in the presence of water and formaldehyde aqueous solutions.

Observing Fig. 4 it can be concluded that the absorbance intensity of both bands decreased with the increase of formaldehyde concentration. The wavelengths of these two bands corresponded to the bands observed with the dried POM coated fibre and the observed intensity changes after immersion in water are due to the formaldehyde-$[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ interaction. However, as it is known that formaldehyde itself does absorb in the UV range, experiments were performed to exclude the possibility of some contribution of pure formaldehyde band to the observed signals. Therefore, an uncoated optical fibre was put in contact with the same formaldehyde standard solutions. No absorption bands could be detected in the whole UV-Vis range of the used spectrometer (250-800nm) with the uncoated fibre in the presence of 6.0 mg L^{-1} of formaldehyde, meaning that the presence of the polyoxometalate membrane was essential to allow the detection of the formaldehyde in solution.

3.4 Analysis of milk samples

3.4.1 Assessment of the optical sensor performance

Fig. 5 shows the UV-Vis spectra obtained with the optical sensor in contact with water, with a milk sample with a formaldehyde content below the limit of detection of the acetylacetone spectrophotometric method (<0.2 mg L^{-1}), and after spiking this milk sample with formaldehyde.

The spectrum of the coated optical sensor in contact with milk, independently of its formaldehyde content showed a significant increase in the band at 261 nm observed in water, while the band at 329 nm maintained its intensity when in contact with milk samples with a formaldehyde content below the detection limit of the acetylacetone spectrophotometric method. The band at 329 nm decreased when formaldehyde was added to milk (spiked sample).
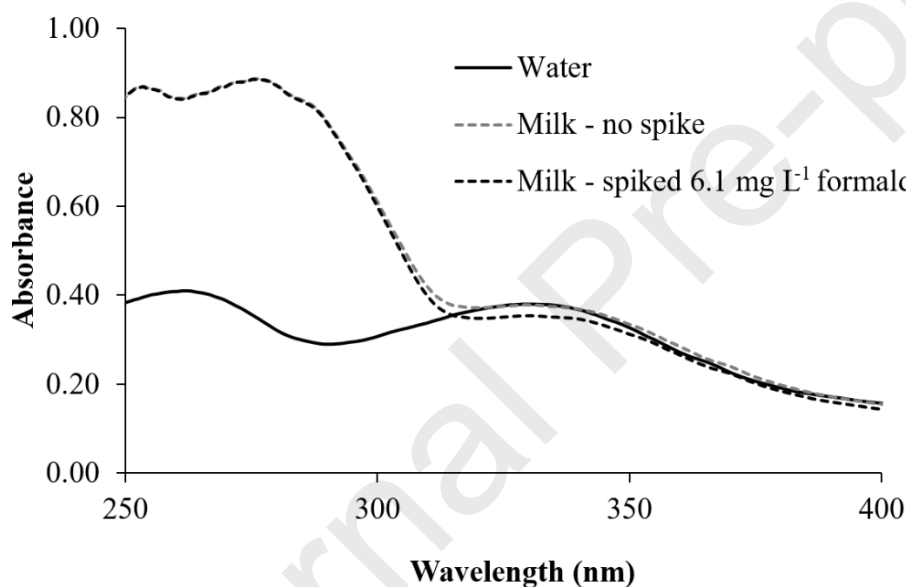
Fig. 5. UV-Vis spectra of the optical sensor in contact with water, with a milk sample with formaldehyde content < 0.2 mg L^{-1} or with the same milk sample spiked with 6.1 mg L^{-1} of formaldehyde.

These observations led to the choice of the band at 329 nm for formaldehyde quantification in milk, in detriment of the band at 261 nm, despite the latter being slightly more intense in the coated dried fibre.

Calibration was performed using the standard addition method to circumvent possible matrix effects. Aliquots of milk were spiked with the diluted standardized formaldehyde solution, to prepare samples with formaldehyde added concentrations from 0 to 6.10 mg L^{-1}. The absorbances at 329 nm were registered after subtraction of the absorbance of water at 329 nm. Minimum least squares regression was applied to 7 spiked samples and formaldehyde concentration in milk was obtained by extrapolation of the regression line.

3.4.2 Analysis of formaldehyde concentration in milk samples

Formaldehyde was quantified in twelve milk samples using the new sensor. For comparison purposes, the formaldehyde concentration was also obtained by the conventional acetylacetone spectrophotometric method. Table 1 shows the formaldehyde concentrations in the samples.

Table 1. Formaldehyde content (mg L^{-1}) in milk samples determined by the new optical sensor and by the acetylacetone spectrophotometric method.

Milk samples	[formaldehyde] mg L^{-1}	
	Optical sensor	**Acetylacetone spectrophotometry**
A UHT, cow semi-skimmed milk A	0.8 ± 0.2	0.9 ± 0.1
B UHT, cow semi-skimmed milk B	1.1 ± 0.3	1.0 ± 0.1
C UHT, cow skimmed milk	0.6 ± 0.2	0.6 ± 0.1
D Cow skimmed powder milk, out-of-date	2.8 ± 0.3	2.8 ± 0.1
E Cow whole powder milk, out-of-date	2.8 ± 0.4	2.5 ± 0.1
F Sheep whole powder milk, out-of-date	2.2 ± 0.3	2.3 ± 1
G UHT, cow whole milk	<0.6	0.5 ± 0.1
H Cow semi-skimmed milk	< 0.6	< 0.5
I Goat whole powder milk, out-of-date	2.0 ± 0.4	2.1 ± 0.1
J Milk for cats	< 0.6	< 0.5
K Sheep whole powder milk	< 0.6	< 0.5
L Goat whole powder milk	< 0.6	< 0.5

Results from the optical sensor were obtained by the standard addition method with a seven point's regression, while acetylacetone spectrophotometry results were obtained by linear regression with seven external standards and triplicate sample readings. Confidence intervals were calculated after the calibration, from the *y*-residuals of the regression lines, the slope and sample readings (Miller & Miller, 2000).

The limit of detection LOD (concentration corresponding to the blank signal plus three times the standard deviation of the blank) for the sensor method was 0.2 mg L^{-1} while the limit of quantification LOQ (concentration corresponding to the blank signal plus ten times the standard deviation of the blank) was 0.6 mg L^{-1}. The sensor method allowed to obtain a linear calibration range between 0.6 and 8.5 mg L^{-1}. LOD for the optical sensor method was not different from LOD of the acetylacetone spectrophotometric method, while LOQ was only slightly lower for the spectrophotometric method (0.5 mg L^{-1}).

The values in Table 1, obtained with the two methodologies, were statistically compared using a paired *t*-test, and it was found that the difference in the mean values between the results of the two methods was not great enough to exclude the possibility that the difference was due to random variability (P=0.05). An *F*-test was running for comparison of standard deviations, and it was found that the standard spectrophotometric method was more precise than the optical sensor method (α= 0.05), in all cases, except for C sample, where standard deviations did not differ significantly (α= 0.05).

By observing the results in Table 1, it can be seen that formaldehyde values in samples A, B, C, F, G, H, I, J, K and L were below the Tolerable Concentration (TC) of 2.6 mg L^{-1}, and therefore in which concerns to formaldehyde, did not present any risk for human consumption. Powder milk samples (D, E, F and I) showed the highest's values for formaldehyde, with two samples, D and E, presenting values above the TC value. These four milk samples exceeded the shelf live, which in the present work may had two effects: one was the obvious alteration of composition, while the other could be the non-compliance with the actual legislation.

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In order to detect any product alteration, sheep whole powder milk samples, out-of-date (F) and within sell-by date (K), were compared by visual inspection. At first sight, inspection reveals that the out-of-date sample F presented a more yellowish colour as well as a more granular appearance, characteristics already reported by others (Thomas, Scher, Desobry-Banon, & Desobry, 2004). These out-of-date milk samples were left forgotten for almost 8 years, under very poor storage conditions, in transparent plastic bags barely closed. During the storage of milk powders, many physicochemical changes, mainly dependent on lactose glass transition, may occur, and they have important consequences on the physical (flowability) and functional properties (solubility, emulsifying, and foaming properties) of the milk powders (Thomas et al., 2004). Also, according to the literature (Nursten, 2005), sugar fragmentation can lead to formaldehyde increase. This could be a possible explanation for the highest formaldehyde content found in all the out-of-date powder milk samples, namely D, E, F and I.

3.5 Reproducibility of the optical sensor

Calibration lines for standard formaldehyde solutions were obtained regularly. Fig. 6 shows the calibration lines obtained along a one-week period, from monday to monday. ANOVA tests performed on results of the relative absorbances obtained for the formaldehyde standards along the six working-days showed that there was no significant difference on values between days (α =0.05), and therefore, no evidence of decomposition or leaching of the sensitive membrane was found during the eight-day period.

■ 26 November

■ 27 November

□ 28 November

△ 29 November

▲ 30 November

○ 03 December

$y = -0.0053x + 0.4906$
$r^2 = 0.9972$

$y = -0.0050x + 0.4891$
$r^2 = 0.9994$

$y = -0.0050x + 0.4924$
$r^2 = 0.9981$

$y = -0.0049x + 0.4900$
$r^2 = 0.9952$

$y = -0.0049x + 0.4847$
$r^2 = 0.9985$

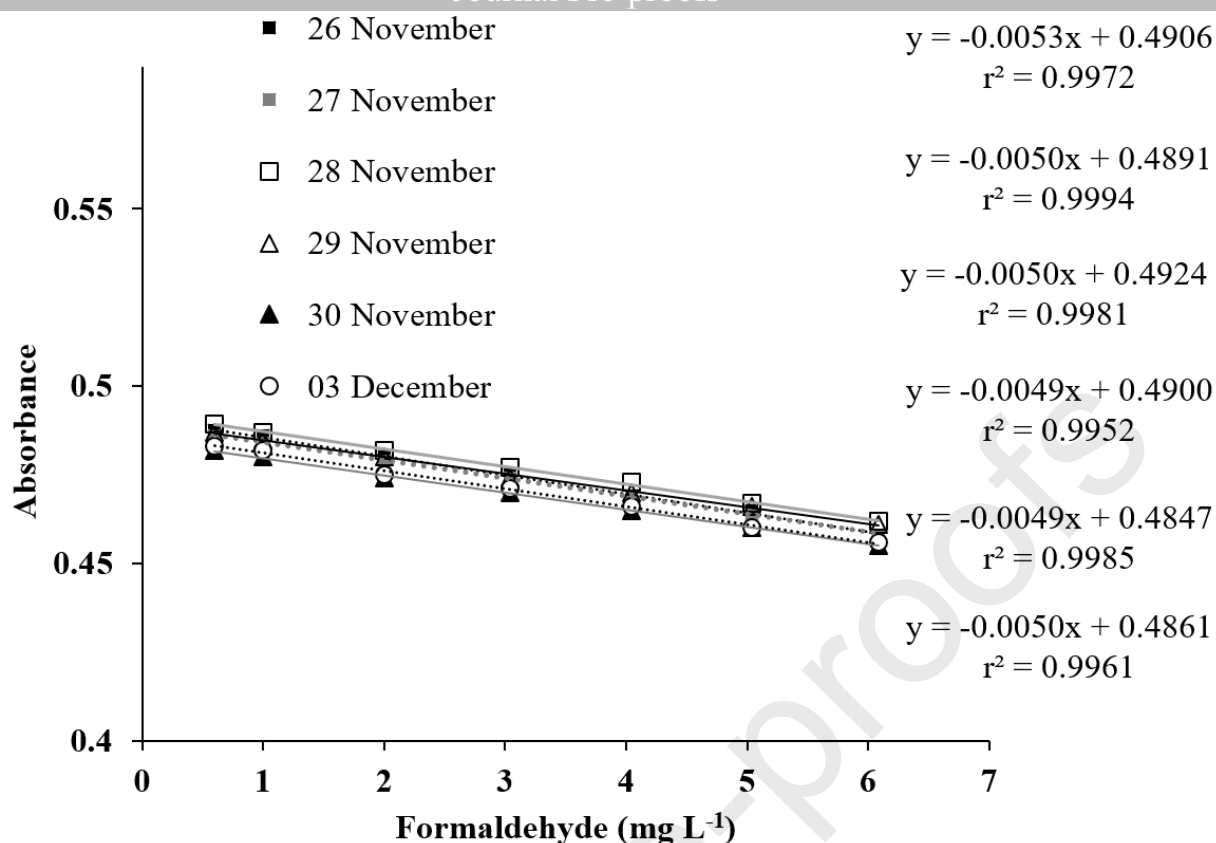$y = -0.0050x + 0.4861$
$r^2 = 0.9961$

Fig.6. Responses of the optrode to formaldehyde standard solutions obtained in different days.

4. Conclusion

A new optical sensor for formaldehyde quantification in aqueous solutions was developed. The sensor was based on an optical fibre coated with a PVC/NPOE membrane containing 1% (w/w) of a specially tailored polyoxometalate compound, $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$. The compound was optically active, and its UV absorbance changed with formaldehyde concentration, while its insolubility in water turned it suitable for application in an optical sensor in contact with aqueous solutions. The new sensor was used to quantify formaldehyde in milk. The limit of detection for formaldehyde with the optic sensor was 0.2 mg L^{-1}, and the limit of quantification was 0.6 mg L^{-1}, very close to the values of the conventional acetylacetone spectrophotometric method. The described methodology has the advantage of not requiring a heating step. The

reliability of the sensor was proved by comparing the analytical results obtained with those found by the conventional spectrophotometric method, without finding significant differences (α =0.05). The sensor kept its sensitivity at least for eight days. Results allowed to conclude that this low cost and easy to use sensor was an effective alternative for formaldehyde determination in milk.

The experimental results revealed that commercial milk and powder milk samples within shelf lives did not present any risk for human consumption, unlike samples that have already expired which presented much higher values, some of them above the legal limits.

Conflict of interest

The authors declare no competing financial interest.

Acknowledgements

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Fig. caption

**Fig. 1.** The experimental setup used with the optical sensor.

Fig. 2. a) SEM image of a PVC/NPOE membrane doped with $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ (1%) and b) EDS analysis of the salt agglomerate on the membrane surface (marked with a cross on Fig.2a).

**Fig. 3.** UV-Vis spectra of the $PVC/NPOE/[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ membrane.

Fig. 4. UV-Vis absorbance spectra of the optical sensor in the presence of water and formaldehyde aqueous solutions.

Fig. 5. UV-Vis spectra of the optical sensor in contact with water, with a milk sample with formaldehyde content < 0.2 mg L^{-1} or with the same milk sample spiked with 6.1 mg L^{-1} of formaldehyde.
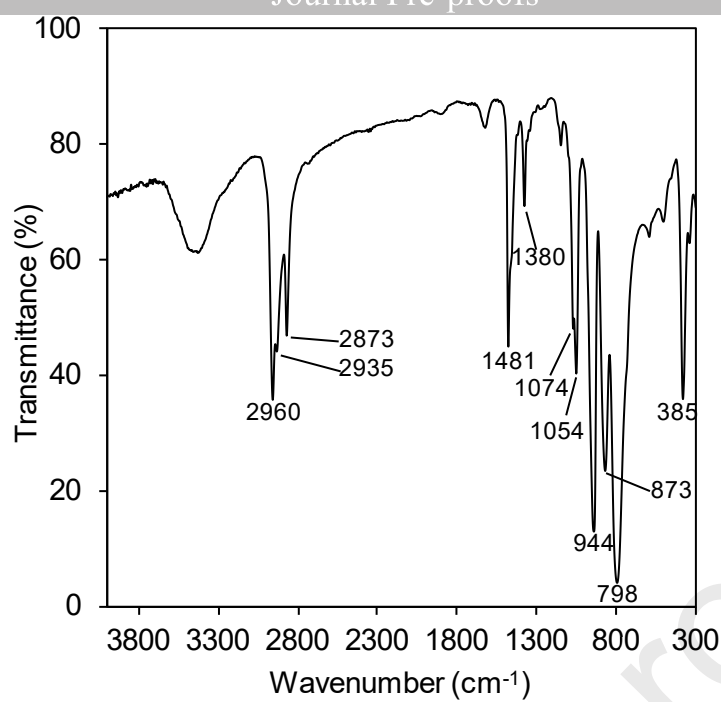
Fig. 6. Responses of the optrode to formaldehyde standard solutions obtained in different days.

Supplementary Material
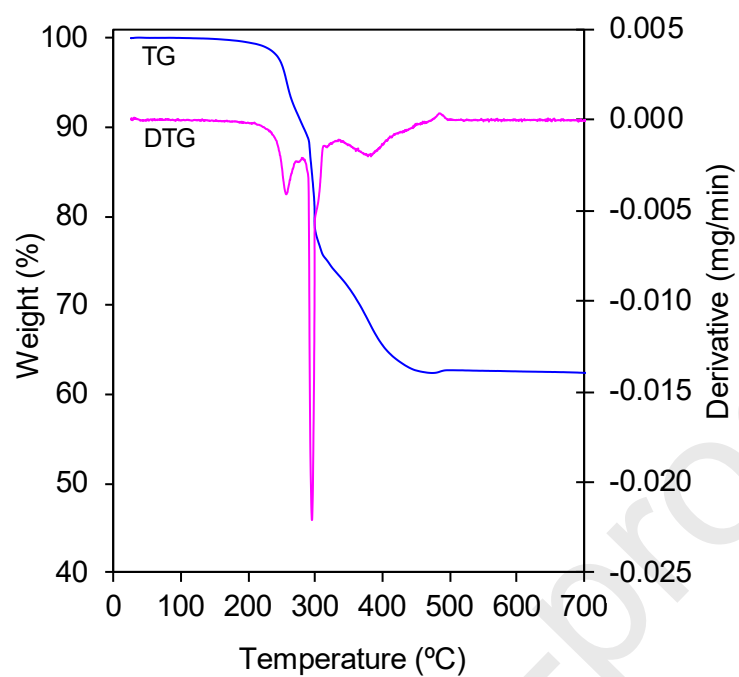
Fig. S1. Thermogravimetric plot and corresponding derivative curve for $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$.
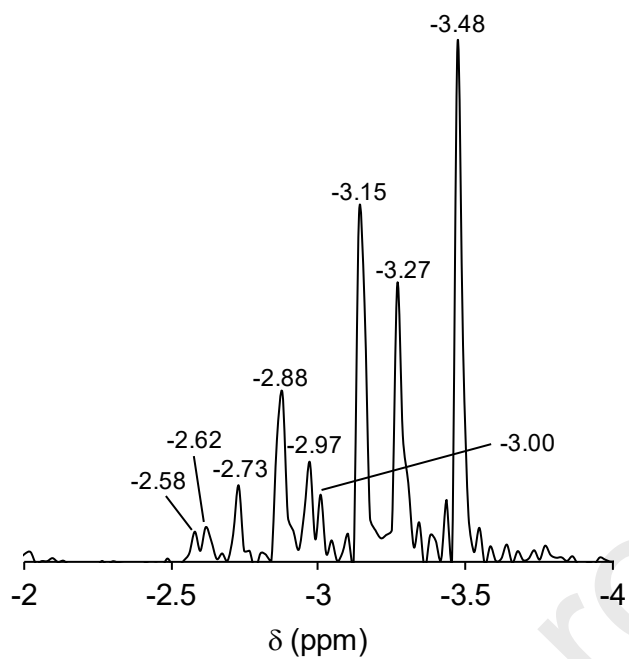
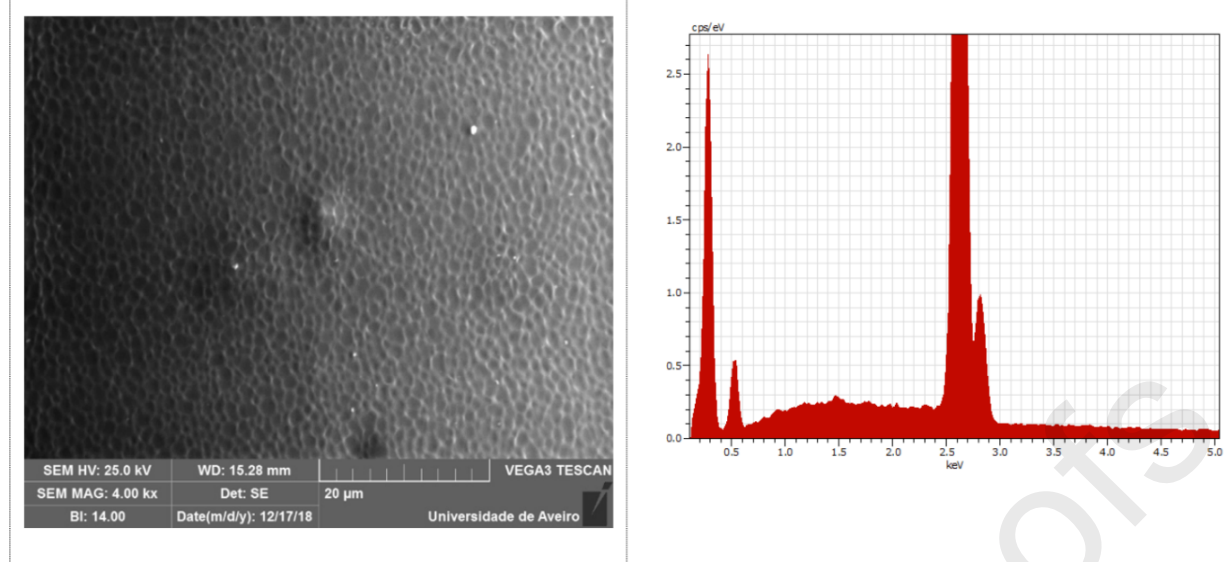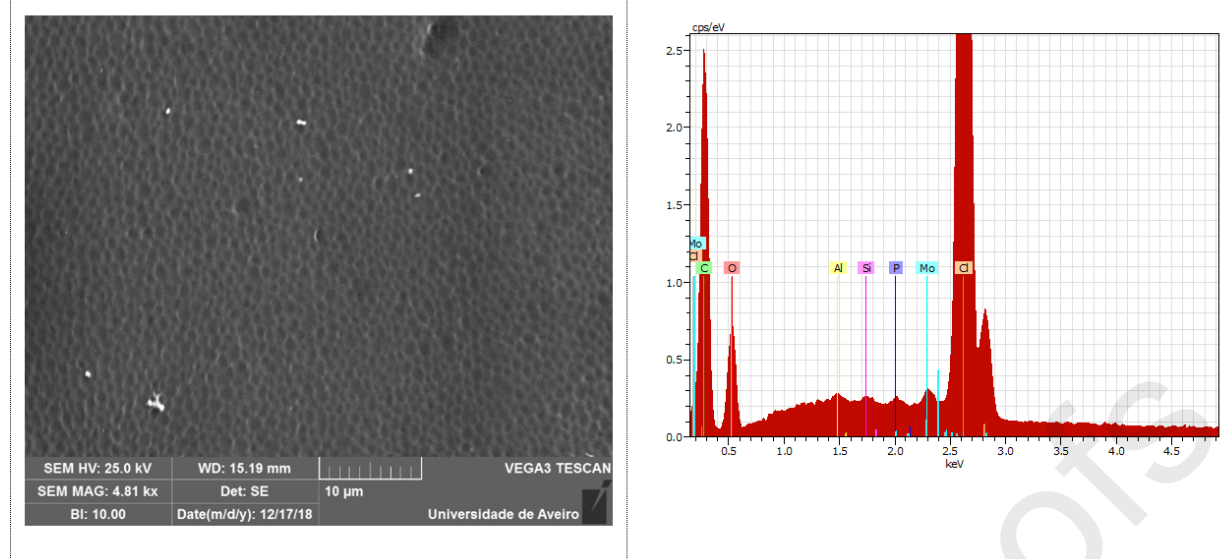**Fig. S2.** ^{31}P NMR spectrum of $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ in dimethyl sulfoxide.

Fig. S3. FTIR spectrum of $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$.

Fig. S4. SEM image of a PVC/NPOE membrane without $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ and EDS analysis.

Fig. S5. SEM image of a PVC/NPOE membrane with $[(C_4H_9)_4N]_4H[PMo_{10}V_2O_{40}]$ (1%) and EDS

analysis.

M. T. S. R. Gomes: Conceptualization, **M. I. S. Veríssimo**: Experiments, **J. A. F. Gamelas and D. V. Evtuguin**: POM synthesis, **A. J. S. Fernandes**: SEM characterization, **M. I. S. Veríssimo and M. T. S. R. Gomes**: Data curation and writing.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

HIGHLIGHTS:

- An optical sensor device for formaldehyde in solution was developed
- It is based on an optical fibre coated with a tailored polyoxometalate (POM) salt
- POM salt changed its UV-Vis spectra in contact with formaldehyde in solution
- The optical sensor showed a quantification limit of 0.6 mg L^{-1}
- The optrode was used to detect quantitatively formaldehyde in milk samples

Table 1. Formaldehyde content (mg L^{-1}) in milk samples determined by the new optrode method and by the acetylacetone spectrophotometric method.

Milk samples	[formaldehyde] mg L^{-1}	
	Optical sensor	**Acetylacetone spectrophotometry**
A UHT, cow semi-skimmed milk A	0.8 ± 0.2	0.9 ± 0.1
B UHT, cow semi-skimmed milk B	1.1 ± 0.3	1.0 ± 0.1
C UHT, cow skimmed milk	0.6 ± 0.2	0.6 ± 0.1
D Cow skimmed powder milk, out-of-date	2.8 ± 0.3	2.8 ± 0.1
E Cow whole powder milk, out-of-date	2.8 ± 0.4	2.5 ± 0.1
F Sheep whole powder milk, out-of-date	2.2 ± 0.3	2.3 ± 1
G UHT, cow whole milk	<0.6	0.5 ± 0.1
H Cow semi-skimmed milk	< 0.6	< 0.5
I Goat whole powder milk, out-of-date	2.0 ± 0.4	2.1 ± 0.1
J Milk for cats	< 0.6	< 0.5
K Sheep whole powder milk	< 0.6	< 0.5
L Goat whole powder milk	< 0.6	< 0.5

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