Flour moisture detection with an europium-based luminescent sensor

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ABSTRACT

In this work, we describe the application of the self-assembled europium complex $\text{1:Eu(III)}$ (where $\text{1:}$ diethyl 8-methoxy-2-oxo-1,2,4,5-tetrahydro-cyclopenta[de]quinolin-3-yl)phosphonate) for the analysis of water content of wheat flour. The methodology herein described represents a robust and accurate way for the detection of small amounts of water in food, providing comparing results with well-established methods such as thermogravimetry and Karl-Fischer titration. Interestingly, as an advantage over other methods, our luminescence-based approach can be implemented in lab-on-a-chip microfluidic devices for real-time and on-line detection of water content of food samples. Additionally, the remarkably long luminescence lifetime of Eu(III) allows the use of state-of-the-art imaging strategies based on PLIM microscopy for the direct visualization of unique water content maps of wheat flour particles.

Keywords: Luminescence, lanthanide, food sensor, moisture, microfluidics, PLIM.
1. Introduction

Water has an important impact on food quality and storage stability as it interacts with food components, influencing their appearance, structure, physical and chemical properties, and stability. Water content affects the taste, aspect, and lifespan of foods. For example, dry ingredients could undergo alterations in consistency of the end product, and excess moisture may cause foodstuffs to agglomerate. Moreover, the rate of microbial growth increases with water content and can result in spoiled food batches, with the subsequent economic loss. Therefore, the analysis of water content is of great importance for the food industry.[1]

Wheat flour is today one of the most important food raw, dry materials, representing the cheapest source of energy and calories. In fact, bread obtained from wheat flour is one of the main energy and nutrient sources around the world, so the control of its quality is extremely important. For instance, wheat flour must be dry enough to inhibit the growth of molds and other undesirable microorganisms. In this sense, farmers and millers aim for a wheat flour moisture content not higher than 14.5% and, therefore, counting with robust methodologies for wheat flour water content detection is imperative.[2, 3] Thermogravimetric methods, which require drying the product at a certain temperature for some time in a drying oven, are currently the standard laboratory technique for water content analysis.[4-7]

However, these methods have problems such as the additional loss of volatile compounds and potential chemical transformations of some of the food components occurring under the analysis conditions. Likewise, strongly bound water may also not be detected at all. Alternatively, a broadly employed and accurate laboratory-based technique to determine water content is the Karl Fischer titration, which consists on the selective reaction of water with a solution of an alcohol, sulfur dioxide (SO₂) and iodine (I₂).[8-11] However, this titration method requires costly equipment, and the necessary chemicals are toxic and expensive. A very sensitive alternative to the Karl Fischer titration is the use of photoluminescent water sensors based on organic molecules or metal–organic hybrid materials. Photoluminescence (PL) measurements present several advantageous features, such as easy preparation, simple operability, high sensitivity, rapidity, and convenient on-site detection.[12-15] However, one of
the main problems when using PL sensors in food samples is the important matrix effect of the samples. Food samples are, on one hand, normally very turbid, due to high content of solids and other sources of light scattering and, on the other hand, these samples may contain potential luminescent interferences. Working in such complex environments requires PL sensors capable of avoiding to a large extent interferences and light scattering. In this sense, PL chemosensors based on the emission of lanthanide ions are of particular interest, due to their high luminous efficiency and narrow spectral widths, and the possibility of applying time-gated PL detection to filter out emissive interferences.[16] Normally, a lanthanide chemosensor requires coordinating the lanthanide ion with suitable organic ligands, including chromophoric groups whose function is transferring energy towards the lanthanide ion (the so-called antenna effect) fostering its emission.[17] Interestingly, the emission from lanthanide ions is very sensitive to quenching by water molecules that can directly interact with the coordination sphere of the metal. This phenomenon has been employed to design specific water sensors.[13] In this field, we have recently reported the discovery of two families of quinolin-2(1H)-one derivatives, which can act as selective Eu(III) and Tb(III) antennas, and proposed their use as highly sensitive sensors of H₂O traces in organic solvents.[18]

Herein we describe the application of the europium antenna diethyl (8-methoxy-2-oxo-1,2,4,5-tetrahydro-cyclopenta[de]quinolin-3-yl)phosphonate (I, Figure 1), whose europium complex, I:Eu(III), showed a limit of detection (LOD) of water in acetonitrile of 0.025 %,[18] to the analysis of water content of wheat flour. The versatility of the newly developed approach was also demonstrated through its application to determine the water content of whole wheat flour. Likewise, we demonstrate the potential use of the sensor in lab-on-a-chip microfluidic devices for a fast and precise on-line continuous determination of water contents of food samples, with reduced waste, reagent, and sample volumes. Moreover, thanks to the exceptionally long PL lifetime of Eu(III), in the order of milliseconds, we have employed PL lifetime imaging microscopy (PLIM) for the direct visualization of wheat flour particles, which allows the innovative imaging of water content maps.
Figure 1. Structure of the Eu(III) antenna 1 and the luminescent self-assembled europium complex 1:Eu(III) employed as a water sensor to determine the moisture extent of wheat flour. In the presence of H₂O, the complex 1:Eu(III) is quenched leading to a significant decrease in the red, long-lived luminescence emission of Eu(III).

2. Results and discussion

2.1. Calibration of the sensor

We previously described that the antenna 1 behaves as a self-assembled selective Eu(III) sensitizer in organic solvents, exhibiting the typical red luminescence of this ion upon excitation at 320 nm.[18] Moreover, the addition of H₂O traces (<2%) to solutions of the 1:Eu(III) complex in CH₃CN turned off the characteristic red luminescence of this cation, which set the background to use 1:Eu(III) as a sensor to determine the water content in organic solvents (Figure 1). Titrations of 54 μM solutions of the 1:Eu(III) complex (2:1 stoichiometry) in CH₃CN with increasing amounts of H₂O (0–2% v/v) were carried out previously, exhibiting a decrease in Eu(III) PL emission with the addition of just 0.005% of H₂O. The red emission was completely quenched with the addition of 2% of water. The quantitative application of the water sensor to food raw materials required estimating the calibration of the sensor response. The titration results fitted quite well to a linear relationship between I₀/I and the % of water content (v/v), where I and I₀ are the PL intensity at 616 nm of the quenched sample solution or the anhydrous blank solution, respectively, following the equation I₀/I = 1 + 4.54 [%H₂O], which may be used as linear calibration for the determination of water content (Figure S1 in the Supplementary Material). This equation allows the interpolation of the relative decrease of intensity of the lanthanide
PL emission in a sample of food to extract the water content, considering that the concentration of water extracted from the food in the test solution may be within the calibration range.

2.2. *Determination of food water content in wheat flour and whole wheat flour*

Since this sensor is capable of detecting extremely low concentration of water, and given the importance of wheat flour as a dry food raw material, we set up a new methodology for water content determination using the europium complex $\text{1:Eu(III)}$. Once optimized, the new approach was also tested by extending the application to whole wheat flour. For that purpose, the free water of the raw food, that was previously dispersed conveniently, was extracted using anhydrous CH$_3$CN. Subsequently, the mixture of the CH$_3$CN food extract and a solution of $\text{1:Eu(III)}$ in dry CH$_3$CN resulted in a considerable decrease in the PL emission intensity of the metal ion (Figure 2). Then, the subsequent interpolation in the calibration response equation provided the amount of water in the sample and allowed working out the water content in the food.

We applied our methodology to wheat flour and whole wheat flour, as models of food raw materials. Initially, two critical factors were optimized, i.e., the appropriate dispersion of the food for a correct water extraction in the anhydrous solvent, and the amount of food to maintain the extracted water within the linear range of the calibration curve of the sensor. We analyzed samples of wheat flour and whole wheat flour taking, at least, 5 aliquots of total mass in the 5-10 mg range. As expected, increasing the mass of the aliquot resulted in a larger quenching of the $\text{1:Eu(III)}$ sensor PL emission and higher $I_0/I$ ratio values, which correlated well with a larger amount of extracted water. In any case, dividing the amount of water in the extract by the mass of the aliquot resulted in consistent relative water content values in the sample, with final values of $10.6 \pm 2.9\%$ for wheat flour and $13.1 \pm 1.7\%$ for whole wheat flour (Figure 3), which are in very good agreement with the tabulated thermogravimetric reference values of $14.0\%$ and $14.0\%$ (Figure 3).[19]
Figure 2. Emission spectra of 1:Eu(III) in the presence of different amounts of (A) wheat flour or (B) whole wheat flour.

Hence, we experimentally compared the results obtained using our luminescent sensor, 1:Eu(III), and the results acquired using two well-established methods for the determination of water extent such as thermogravimetry and the Karl-Fischer coulometric titration. Thus, the thermogravimetric method yielded water content values of $11.2 \pm 0.1\%$ and $11.7 \pm 0.1\%$ for wheat and whole wheat flour, respectively, while Karl-Fischer titration resulted in water content values of $12.1 \pm 0.3\%$ and $11.7 \pm 0.2\%$ for wheat and whole wheat flour, respectively (Figure 3). Importantly, these results are in very good agreement with the ones obtained using our lanthanide-based sensor (Figure 3), 1:Eu(III), underscoring the efficacy of our methodology as a robust and precise approach for detecting trace amounts of water in wheat flour.
Figure 3. Moisture (% w/w) of wheat flour and whole wheat flour obtained by luminescence (white), Karl-Fischer (light grey), thermogravimetry (dark grey) and reference value (black) from reference [19].

To test the performance of our methodology to detect variations of water content, i.e. gain or loss of water, we changed the water content by drying or adding water to wheat flour. The PL emission intensity of the 1:Eu(III) sensor in the CH$_3$CN extract was recorded from 5-10 mg of wet wheat flour (external addition of spiked water). Additionally, the wet wheat flour was oven-dried at 120 °C for 1 h and kept in a desiccator prior analysis. We termed this sample ‘wet&dried’. As expected, the wet wheat flour resulted in an almost total quenching of the PL emission of the 1:Eu(III) sensor. Interpolation in the calibration curve and normalization to the original flour weight yielded the water content value of 14.3 ± 0.5 % for the ‘wet&dried’ sample. Interestingly, the ‘wet&dried’ sample recovered, after desiccation, showed similar water content levels than the original material. This confirms that mild drying, at 120 °C, can be sufficient to remove excess water, loosely adsorbed.

2.3. Estimation of water activity ($a_w$)

Water activity ($a_w$) is a key parameter in food science to understand the availability of water in food products, allowing us to predict the probability that some degradation reactions of food may take place. The water activity is correlated with the moisture of some food in equilibria with their environment/atmosphere from the well-known isotherm models, such as BET or GAB.[20] For this reason, once the moisture extent was determined, next we correlated these moisture values with the
corresponding water activity values. For that purpose, wheat flour was employed as a model, since there are reported several well-established water isotherms for this food.[21, 22] Thus, values of moisture content obtained by the previous method (% w/w) were converted into water content per mass of solid food, χ (g of H₂O/g dry solid), in order to associate them to the isotherm reported by Martin-Santos and coworkers,[21] fitted to the GAB model. The water sorption isotherm shows three different parts, in which the same amount of increase in moisture results in different water activity values, for example, an increase of moisture above 0.3 g of H₂O/g of dry solid results in a significant gain of water activity. This experimental isotherm, fitted to the GAB model, was then employed to extract water activity values from χ (Figure 4). We used the calculated water content for the different samples of flour, i.e., wheat flour, whole wheat flour and ‘wet&dried’ wheat sample. As shown in Figure 4, we estimated a higher water activity for whole wheat flour (0.077) than for wheat flour (0.057) and a water activity value of 0.088 for the ‘wet&dried’ sample.

![Figure 4. Water adsorption GAB isotherm (line) for wheat flour according to Martín-Santos and col.[21] Points represent the water activity obtained from experimental χ values, using the 1:Eu(III) sensor, for wheat flour (red), whole wheat flour (light blue), and ‘wet&dried’ wheat flour sample (orange).](image)

2.4. Estimation of water content in a microfluidic chip

In the food industry, real-time, continuous-flow and in-situ analysis of certain analytes is an important tool for rapid and fast quality control, especially when miniaturized. The great benefit of
physically scaling down analytical systems is not only a reduction in size, reagent consumption and waste, but rather an improvement of the analytical performance.[23] Hence, lab-on-a-chip systems based on microfluidics have been successfully employed as tools for the detection of a variety of analytes. Therefore, we designed a microfluidic device (see Figure 5 and Figures S2-S4 for details on the design, preparation, characterization and implementation of the mixer chips) to perform real-time continuous-flow water content measurements using the luminescence emission of the 1:Eu(III) sensor. The microfluidic chips were fabricated in OSTEMER by injection molding on a polydimethylsiloxane (PDMS) mold fabricated by a low-cost approach based on standard soft lithography techniques.[24] The microfluidic chip (Figure S3 in the Supplementary Material) comprises two inlets for reagent injection, and a butterfly-type passive micromixer allowing for submillisecond mixing times, as reported elsewhere.[25] For our experiments we placed the 1:Eu(III) complex (loaded in anhydrous CH₃CN; 20 µM of 1 and 20 µM of EuCl₃) in one of the channels and the other was loaded with either the solvent (anhydrous or non-anhydrous for calibration) or a solvent extraction of wheat flour (Figure 5). The very fast mixing in the chip allowed for real-time measurements of fluctuations in the PL emission of the 1:Eu(III) probe, providing immediate detection of small changes in water content. The micromixer chip was incorporated into a confocal microscope to directly send the excitation light (a pulsed, 375-nm laser) into the channel of the mixer, and collect back the Eu(III) luminescence signal, sent to a point detector with a red-centered bandpass filter (630/60 nm) (see the SM and Figure S5 for further details). This setup also allowed us to image the microchannels as diagnostics, confirming the excellent mixing efficiency. The PL emission inside the microchannels showed absence of significant autofluorescence when the testing chamber had no probe and a strong emission when the channel was filled with the europium complex 1:Eu(III).
Figure 5. Schematic representation of the (A) microfluidic-based system for the detection of water content in wheat flour and the (B) employed home-assembled microfluidic chip.

For the use of the micromixer chip for real-time water content measurements, we registered PL emission time traces at different points within the mixing channel to optimize the signal. Figure 6 shows normalized PL time traces ($I/I_0$) from the $1$:$\text{Eu(III)}$ probe when mixed with anhydrous CH$_3$CN, or CH$_3$CN spiked with 1% or 5% of H$_2$O, clearly showing the quenching of the red emission from $1$:$\text{Eu(III)}$ caused by water molecules. We calibrated the response of the $I/I_0$ parameter towards H$_2$O content in the microfluidics experiments, given instrumental and experimental differences between the fluorometer and the confocal microscope that prevent using the same calibration equation. For the application with food extracts, the $I/I_0$ time trace was directly converted into water content traces (considering the dilution factor upon mixing), which illustrate the possibility of real-time determination of water content fluctuations from food extracts (Figure 6). We performed this measurement with CH$_3$CN extracts from
wheat flour, as explained in the previous section, and obtained an average value of $8.4 \pm 0.3$ % of water, in good agreement with the results obtained off-line using a cuvette. Remarkably, the implementation of this system showed its ability to detect real-time and continuous-flow traces of moisture in wheat flour extracts, demonstrating its promising potential in lab-on-a-chip technologies.

**Figure 6.** A) Normalized intensity traces ($I/I_0$) obtained in the microfluidics device, after fast mixing, for the 1:Eu(III) probe in dry CH$_3$CN (black) and containing 1% (red) or 5% (blue) of water, and that in the CH$_3$CN extract (3 mL) from 1 mg of wheat flour (green). B) Percentage of water content in 1 mg of CH$_3$CN extract from wheat flour measured in real time. Shaded green area represents the standard deviation from the interpolation of the values in the calibration. The average value over the 1 s time trace was $8.4\pm0.3$% (gray dashed line).

2.5. **Water content maps in wheat flour particles by PLIM microscopy**

Owed to the certainly long PL lifetime, $\tau_{PL}$, of the emission of lanthanides ions and, specifically, that of the 1:Eu(III) complex (0.55 ± 0.04 ms in CH$_3$CN) and its exquisite sensitivity to quenching by the presence of water molecules, which decreased its $\tau_{PL}$ from 0.49 to 0.14 ms,[18] we decided to use
PL lifetime imaging microscopy (PLIM) to directly visualize wheat flour particles, of micrometer size (Figure S5 in the Supplementary Material). Importantly, this imaging approach allows a time-gated analysis to filter out all potential sources of natural background fluorescence and scattered light, and therefore increasing the sensitivity, signal-to-noise ratio, and reliability of the detection.[16] The sensitivity of $\tau_{PL}$ towards water content allows building up unique water content spatial maps.

Herein, we tested this concept by imaging wheat flour particles, containing different amounts of H$_2$O (natural content, 1% or 10% of added water, or dried particles), suspended in anhydrous CH$_3$CN with the 1:Eu(III) probe (20 $\mu$M of 1 and 20 $\mu$M of EuCl$_3$). PLIM images (Figure 7) showed the characteristic Eu(III) PL emission with millisecond lifetime values concentrated in micrometer-sized particles that were attributed to flour particles. This enhanced luminescence on the particle surface arose from adsorbed 1:Eu(III) complex, as evidenced when compared in controls with and without flour (Figure 7A and Figure S6 in the Supplementary Material), and the fact that depending on the confocal plane focused we observed hollow (the confocal plane cut the particle, showing only the outer surface) or full flour particles (the topmost or bottommost surface of the particle was imaged). For instance, as shown in Figure 7B, PLIM images of wheat flour particles showed strong adsorption of 1:Eu(III) complex with an average PL lifetime $\tau_{PL}$ in the order of 0.31 ms, which was distributed in two lifetime populations, the main at ca. 0.30 ms, and a secondary around 0.50 ms, that suggested that 1:Eu(III) complex was present in different hydration degrees. The changes in the average $\tau_{PL}$ values were followed quantitatively in the lifetime distributions as well as in the overall PL decay traces (Figure S7 in the Supplementary Material). To confirm that PL emission was indeed coming from the 1:Eu(III) complex, we collected the emission spectrum at different points within the flour particles, using the hybridization of a spectrograph to our confocal PLIM microscope (see SM for further details).[26] and found unequivocally the spectral shape of Eu(III), that is the narrow emission bands at 593, 616, and 703 nm, correlated with almost total quenching of the antenna 1 emission, as expected for efficient energy transfer towards the lanthanide ion.
Figure 7. Representative PLIM images and their $\tau_{PL}$ distributions acquired in the Eu(III) channel from pixels of the images and emission spectra of selected points collected by irradiating the marked points of samples of A) EuCl$_3$ and wheat flour particles; B) 1:Eu(III) complex and wheat flour particles; C) 1:Eu(III) complex and wheat flour particles with 1 % of externally added H$_2$O; D) 1:Eu(III) complex and wheat flour with 10 % of externally added H$_2$O; and E) 1:Eu(III) complex and dried wheat flour particles.
Interestingly, the addition of 1% of H₂O to wheat flour particles prior to the suspension in CH₃CN resulted in a significant decrease in the average $\tau_{PL}$ of the complex, from 0.31 to 0.25 ms (Figure 7C), whereas the addition of 10% of H₂O caused a complete extinction of the complex PL emission (Figure 7D). Remarkably, when we removed the moisture of the wet wheat flour particles (10% of H₂O) by oven-drying at 120 °C for 1 h, PL emission of the 1:Eu(III) complex in the flour particles was restored (Figure 7E), recovering $\tau_{PL}$ values around 0.41 ms. In summary, obtaining these water content maps represents a novel concept to gain insight about the microstructure of wheat flour particles, which can be extended to other fundamental investigations leading to further quality assessment and product innovation advances.[27]

3. Conclusions

We have demonstrated the quantitative relation of Eu(III) luminescence quenching and water content in CH₃CN solutions, using our 1:Eu(III) probe. For very low amounts of water there was a linear relationship between relative luminescence intensity ($I_0/I$) and water content. The sensitivity of 1:Eu(III) to small amounts of water content makes it useful for bench applications, to detect small amounts of water extracted from wheat flour. The methodology herein described represents a robust and accurate way for the detection of water in wheat flour, and to determine the corresponding water activity. Moreover, it provided comparing results with well-established approaches such as thermogravimetry and Karl-Fischer titration, which are inherently destructive and may lead to undesirable reactions, changing the nature and mass of the food.[28] Remarkably, our method can be implemented in lab-on-a-chip microfluidic devices for real-time and on-line detection of water contents in food extracts. Moreover, the exceptionally long PL lifetime of Eu(III) permits the employment of state-of-the-art imaging strategies based on PLIM microscopy for the direct visualization of unique water content maps in micrometer sized flour particles.

Conflict of interest

The authors have no conflicts of interest to declare.
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Supplementary Material

Supplementary material to this article can be found online at

References


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