

Optimizing Novel Interference Film Sensor for Food Degradation

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ABSTRACT

An optical thin film sensor chip able to detect the decay of food through a specific color change is presented. The design of the sensor relates to the phenomenon of “anomalous absorption”, which can best be described as a thin film enhanced absorption. A metal cluster film positioned at a well defined distance to a smooth metal surface shows that the minimum of spectral reflectivity strongly depends on the thickness of the interlayer: This setup represents a special kind of reflection interference filter. In such a sensor setup we have integrated a biodegradable polymer which is degraded by the same enzymes and at the same rate as food decay will happen. The degradation of the polymer results in reduction of the film thickness and thus in a specific change of the color.

Keywords: optical thin film sensor, anomalous absorption, interference, food spoilage, biodegradable polymer

1 INTRODUCTION

In the recent years, the combination of knowledge in electrochemistry, biochemistry, physics and integrated circuit silicon technology made it possible to provide highly specific, sensitive, selective, accurate and reliable micro-biosensors [1,2,3]. The biosensor field is growing so rapidly and has become so diverse that it is impossible to comprehensively cover the entire field in this introduction. It encompasses an extremely diverse number of methods and applications. In the production of goods environment biosensors can be used for on-line measurement of critical intermediates or products; in the medical field, they can be used for rapid and economical determination and monitoring of metabolites, drugs or hormones, never the less biosensors can be used as micro sensing and control devices in the service of environmental, agricultural and food-processing applications. If a biosensor is defined as a device with a biological recognition element built in (physically attached or confined) and this is the primary selectivity element, the freshness sensor for food we want to represent herein is a typical biosensor. This freshness sensor for food is irreversible and therefore suitable for single use only. It is not activated until it gets in contact with the meat. The sensoric approaches established so far have made use of indirect parameters like pH and temperature. These parameters do not reflect the real quality of the meat to be tested. Monitoring the pH gives only indirect information and most tests are reversible, and therefore generally unapt to contribute to the safety of the

meat supply chain against willful or neglectful corruption of the sensor function. Monitoring the cold chain through chemical or electronic sensors is expensive and yields indirect information as well, strongly raising the risk of creating a lot of additional wasted foods. The laboratory tests as used and especially if combined with each other yield very reliable results but are very expensive and time consuming. The aim of our novel approach has been to create a sensor that provides reasonable sensitivity and selectivity to indicate the “best use before” period combined with a memory effect that cannot easily be corrupted.

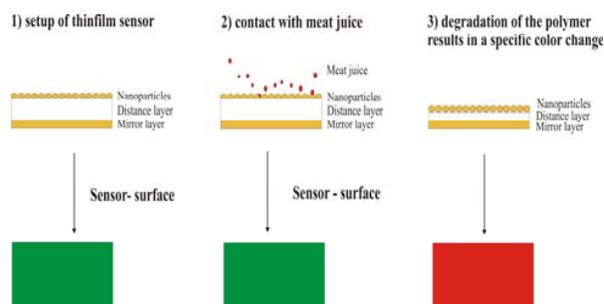


Fig. 1: Scheme of sensor principle

Another big advantage of this new sensor system is the cost efficiency for its production. Every step of the sensor production has been carried out on industrial testing equipment and with coating procedures like PVD and gravure printing. These production methods guarantee very good economies of scale and thus low cost per unit.

The freshness sensor for food is an optical thin film sensor with an integrated biopolymer, which is degraded by the same enzymes at the same rate like food, as e.g. meat (Fig.1). Due to the special optical behavior of a metal island film and due to the thin film set up, this system shows a characteristic spectral reflection behavior, strongly dependent on the thickness of the transparent interlayer [4]. The optical property of metal island films, necessary for our application, is the so called “plasmon absorption”, a strong, broad-band absorption in the visible, which is due to the confinement of the conduction electron plasma in nanometric particles. This is in contrast to the unconfined electron movement in an extended metal, responsible for strong, unspecific reflectivity, well known as metallic glance. An absorbing thin film positioned at a defined distance to a metal mirror represents a special kind of reflection interference filter. At an appropriate distance of the absorbing layer to the mirror, fields reflected by the

mirror have the same phase at the position of the absorbing layer as the incident fields and, thus, by this feedback mechanism the effective absorption coefficient of the absorbing layer is strongly enhanced. This combination of the two phenomena plasmon absorption and optical interference is generally called anomalous absorption. Fig. 2 shows the visual impression obtained by observation of the reflected light upon diffuse white-light illumination of the layer system used in our sensor set up in the interlayer optical thickness range 0 – 490 nm (optical thickness = geometrical thickness \times effective refractive index) and some corresponding, measured reflection spectra.

The authors and other research groups have developed a number of sensoric applications based on the principle of anomalous absorption targeting amongst other parameters

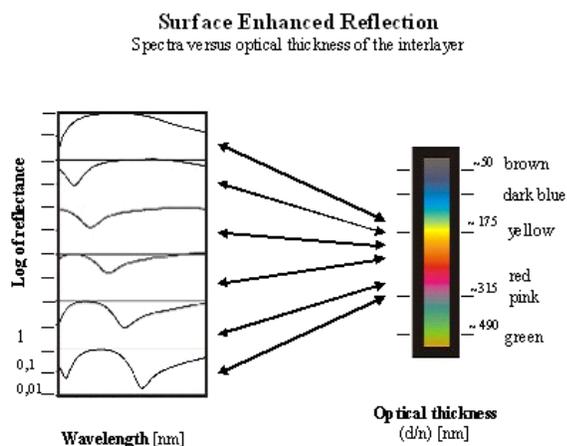


Fig. 2: Visual impression obtained by observation of reflected white light by the freshness of food and reflectance spectra. Range of the interlayer thickness 0-490 nm in steps of 35 nm

pH, humidity and salt concentration [5]. In a number of these publications the reversible thickness change of the sensor layer has been transduced into an optical signal. In the so-called MICSPOMS sensors a polymer with shrinking and swelling properties is integrated as the distance layer. The response of MICSPOMS on ionic strength is fully reversible and due to the direct exposure of the very thin swelling polymer layer to the analyte is so fast that no delay of response can be observed visually [6]. In this paper we report the setup of the sensor and our first results we made with the detection of different enzymes and in comparison with different meat juices. Unlike MICSPOMS our sensor response is not reversible. The irreversible degradation of the polymer results in a decrease of sensor layer thickness and this creates a permanent color change.

2 MATERIALS AND METHODS

2.1 Materials

We used Esterase from horse from Fluka and used this enzyme in different concentrations in 0.1M Tris-HCl pH 8. For our experiments with meat juice we bought packaged

fresh meat from pork from local retailers in Vienna (Billa). We then aliquoted the meat juice in 500 μ l Eppendorf tubes in 50 μ l aliquotes and froze them in fluid Nitrogen. Adjacent we stored the frozen meat juice at -20 °C. Every experiment was carried out with 0.1M Tris-HCl buffer and ddH₂O as a negative control. Our substrate for the thin film setup was PET foil from Ineos Films (UK). For all necessary washing steps we used ddH₂O. Our degradable interlayer polymer is PHB (Polyhydroxy butyric acid) from Biomer (Germany) in Chloroform as a solvent for printing applications. As a crosslinker for the sensoric polymer we used industrial grade poly aromatic isocyanate from Bayer (Germany). We tested different metals as the mirror layer: i.e. Gold and Aluminum.

2.2 Methods

2.2.1 Coating

Gravure Printing, also known as Intaglio printing, is accomplished by cutting or engraving and etching various sizes or depths of minute cells (or wells) below the surface of a plate or cylinder to form the ink film. The cells are flooded and loaded with ink, the excess ink is scraped off the surface of the plate by a doctor blade, and the ink left in the cells is transferred to the substrate. The depth and size of each cell determines the amount of ink that is transferred to the printed surface. The nature of the process permits a heavy laydown of ink, which accounts for the rich, saturated colors typical of the gravure process. We used a semi automatic gravure printer for laboratory use from Erichson (Germany).

We prepared a 5% PHB (Polyhydroxy Butyric Acid) solution in HCCl₃ with 0,025% w/v crosslinking agent and printed this solution with (20 m / minute) on top of the mirror layer.

Dip Coating: Another possibility to bring the polymer on top of the mirror layer is dip coating and spin coating. Dip coating refers to the immersing of a substrate into a tank containing coating material, removing the piece from the tank, and allowing it to drain. As a dip coater we used a self made dip coater made from building blocks from Fischer Technik. This technique gave us the possibility to dip coat with a defined speed and with a defined immersing angle. To coat the mirror layer with PHB by dip coating we also prepared the 5% PHB solution in HCCl₃ with the same concentration crosslinking agent. Then we attached the substrate (PET with mirror layer) onto a support (glass slide) and immersed it with consistent velocity into the solution. There we leaved it for 20 sec and then we pulled it out with the same velocity. Both methods today are widely used for the mass production of nanometric thin films. Generally the coated pieces can then be dried by force-drying or baking.

Sputter Coating: To compare the success of the different coating processes we applied nanoparticles by Sputter Coating, generating the anomalous absorption and visualizing the homogeneity of the printed layers. The mirror layer and the nanoparticles were sputter coated with an Agar sputter coating system for electron microscopy. We sputtered Au with a Argon Plasma at 0,08 mbar for 10 sec and generated a film thickness for the nanoparticles

from approximately 4 nm. For the mirror layer we also sputter coated Au under the same conditions but prolonged sputter time to 60 sec.

2.2.2 Sensor-Setup

The here presented thin film sensor has a sensoric layer with a maximum thickness from 50 to 500 nm and, thus, shows very fast response. The decomposition of only a few links between the polymer chains causes instability to the degree that the sensoric layer is destroyed upon immersion in aqueous solutions. As the metal island film is highly permeable for the analyte the sensor layer is directly exposed to the analyte. For a tuning of interlayer thickness there is one important consideration: Like all interference colors the visual perception of the sensor surface is strongly dependent on the distance layer thickness. Thus the visually observed color can be used to determine the actual thickness of the polymer layer. We have manufactured sensors ranging from blue, the yellow and green with sensor layer thicknesses corresponding to 80, 120 and 150 nm of mass thickness. In order to determine the actual thickness of the distance layer more exactly we will carry out further experiments employing AFM.

In order to provide sensors for food sensing, the sensor setups have to be stable over prolonged incubation with meat juice. The typical incubation conditions along the meat supply chain range from -20°C up to 40°C. The rate of enzyme activity is following Arrhenius law both for meat degradation and for sensor setup.

2.2.3 Test Procedure

For a functional assay we prepared different solutions as described in the scheme (Tab.1), every solution was

1. Meat juice	1µl
2. Meat juice 1:2 with buffer	1µl
3. Meat juice 1:2 with buffer with 5mg/ml Esterase final concentration	1µl
4. 10 mg/ml Esterase in buffer	1µl
5. buffer	1µl
6. ddH ₂ O	1µl

Table 1: Pipetting scheme

applied twice in two adjacent dots. We pipetted 1µl from every solution on top of the sensor surface. We then incubated the sensors in a humidity chamber for 6 hours at 4°C. After the incubation time we washed the sensor surface with ddH₂O and dried them under an airstream.

2.2.4 Documentation and Quantification

For our documentation we used a flat bed scanner HP Scanjet 4890. Such reflecting films like our sensors can be scanned very good and edited as jpeg.-pictures, if a kind of scatter filter is placed between the sensor and scanner surface. We used a parafilm as a scatter filter. For a quantitative analysis we used a freeware program Image J

(courtesy of NIH), which can count the amount of pixels per picture. Thus, we were able to compare and to quantify different enzyme concentrations.

Also we were able to compare fresh and old meat juice and analyze the resulting data.

3 RESULTS

In this work we present a new kind of food sensing. Until now only changes in pH were measured or a break in the cold chain. This makes it possible to estimate some risks, but not to detect the spoilage of food. For the first time we were able to show the spoilage of meat with a color change in a polymer film embedded in a thin film sensor. This makes it possible to follow the deterioration of food in real-time, e.g. in a package.

3.1 Optimizing the Setup

First we compared the different coating methods to produce a thin, reproducible and homogeneous polymer film as a interlayer in our sensor set up. The dip coating process of the polymer results on the other hand in an inhomogeneous and not very reproducible film thickness. The advantage of such a coating procedure is the ability to use small sample volumes and to get fast results. Then a gravure printing process was established to coat the mirror surface with the PHB. Solutions of various concentrations of PHB in HCCl₃ and 0,025 % w/v cross-linking agent were applied by a printing proofer. Different speeds and different pressures were tested. Fig.3 should give an impression about the different colors as a result of different interlayer thickness.



Fig. 3: different polymer concentrations results in different color of the sensor surface: 1: 5% w/v PHB; 2: 6,25% w/v PHB; 3: 8,4% w/v PHB, all in HCCl₃

The advantage of the gravure printing process is the high reproducibility and also the possibility to produce different colors. The higher the quality of the printing plate the more precise the printing result will be. After the deposition of the Au nano-particles the sensors are ready to use. No additional step is required.

3.2 Proof of Principle

Sensor set up was as follows: on top of a PET Film as substrate we sputter coated ca. 30 nm Au as a mirror layer. The polymer 5% w/v in HCCl₃ with 0,025% cross-linking agent was printed and then the Au nano-particles as a very thin film with 4 nm were sputter coated. For proof of the basic sensor function mixtures from meat juice and enzyme were prepared and compared to various negative

controls. The aim was to detect a degradation of the polymer interlayer by the meat juice and the enzyme.



Fig. 4: Degradation of the interlayer with meat juice, diluted meat juice and mixtures from meat juice and enzyme can be seen very clearly (1-3). (4) shows the degradation of the polymer by the enzyme in buffer, (5&6) are the negative controls buffer and ddH₂O.

The color change shown in Fig. 4 is a result of enzymatic degradation of the sensor. This degradation is highly selective and very sensitive; no degradation through the negative controls took place. As it is seen with the naked eye, the solution 3, a mixture from both enzyme and meat juice has the highest signal. Buffer and Water give no signal and the enzyme 5mg/ ml in 0,1M Tris-HCl buffer results in the smallest signal. These results could indicate that there are more than one enzyme present in the meat juice.

4 CONCLUSIONS

In this work we could present a freshness sensor for food for the first time, which is able to show the deterioration of fresh meat with a color change visible for the naked eye in real time.

We have achieved a number of steps described herein: the optimization of lab manufacturing setup, including an optimized printing process and optimized vacuum deposition process. Both procedures were optimized at the available devices, still not for large scale production but for prototyping. Also we could optimize the analytical evaluation with the optical results. We could show a quantitative analysis of degradation of the interlayer with different concentrations of lytic enzymes. An important point to describe the sensor function is the application in real time measuring format. The degradation of the polymer takes place simultaneously alike the deterioration of the meat.

The freshness sensor for food is able to adapt for different potential commercial applications: to enforce in store quality control, or to give the consumer a transparency of meat freshness and the possibility for self-control of the fresh meat at home.

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