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Determination of stability characteristics for electrochemical biosensors via thermally accelerated ageing

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ABSTRACT

Biosensors are devices that are prone to ageing; this phenomenon can be characterized as a decrease in signal over time. Biosensor stability is of a crucial importance for commercial success and as biosensors are presently being applied to an increasing and variety of applications. Stability characteristics related to shelf life, reusability and/or continuous use stability are often poorly investigated or unreported in literature, yet are important factors. Instability or ageing can be accelerated at an elevated temperature; Arrhenius (exponential) and linear models were investigated in order to propose a novel method for rapid ageing characteristics determination. Linear correlation proved more suitable with higher coefficients of determination than exponential correlation. Degradation rate is linearly dependent on temperature and by utilizing the proposed models, long term shelf life of a biosensor can be determined in 4 days and continuous use stability in less than 24 h. Reusability studies are found to correlate poorly due to the unpredictable nature of biosensor handling. Basic constructed screen printed electrode glucose oxidase biosensors were used as a model biosensor in order to propose models for shelf life, reusability and continuous use stability.

1. Introduction

Biosensors are currently being exploited in several very different fields and applications ranging from basic biochemical/environmental analysis [1–4], food [5,6] and industrial processing, biosecurity [7], pharmaceutical [8] to personalized diagnostics [9–12] and medical research [13–15], with more than a few commercial products available on the market [16]. Stability of a biosensor is of a crucial importance, be it a biosensor for monitoring malolactic fermentation in wine [17], magnetostrictive detection of *Salmonella typhimurium* [18] or blood glucose monitoring [19]. The view on ageing is industry and product specific – a battery producer would view ageing as a decrease in energy capacity retention [20], food and cosmetic industry would refer to bacterial growth or ingredient oxidation [21], while in the pharmaceutical industry it is not only the depletion of the active ingredient but also the occurrence and increase in the concentrations of impurities that is considered as an effect of ageing [22]. In the face of the widely available reports on the stability of the biological components of biosensors i.e. for enzymes [23,24] and antibodies [25–27], less than a couple of these collective reports feature the stability of biosensors as a whole system.

Biological products of any kind are prone to ageing and biosensors are no exception. What is more, the ageing of a biosensor, or product stability, is an important limitation to commercial success [28]. Biosensor ageing is referred to as a decrease in sensitivity over a period of time. Ageing mechanisms of a biosensor or decay in sensitivity are complex and affect each layer and reagent used. Biosensor ageing is therefore the sum of total changes in the functionality of the complex, be it the biological component, be it an enzyme [23,24] or antibody layer [25–27], signal mediator (e.g. Prussian Blue [29,30]) or protective membrane (e.g. Nafion) [31]. The purpose of present work is not to investigate the ageing phenomena and mechanisms due to the complexity of the system, but rather provide a simplistic protocol and model for quick assessment of ageing characteristics for biosensor devices.

Arrhenius was among the first scientist that mathematically recorded and physically justified the effect of temperature on chemical and biochemical reactions, including protein denaturation, oxidation, hydrolysis and other phenomena and mechanisms of ageing. Currently, Arrhenius type extrapolation for rapid tests at elevated temperatures is the most commonly used method to determine long time, low temperature ageing stability of a certain product [32].

Despite the frequent use of the Arrhenius model for stability studies, it is often criticized as the exponential fitting is rarely optimal.

Abbreviations: SPE, screen printed electrode; WE, working electrode; RE, reference electrode; CE, counter electrode; PB, Prussian blue; m, mass; V, volume

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In the food industry, Schaal oven stability test is often conducted [33–37]. Relying on the assumption of a linear correlation of accelerated ageing e.g. a test object incubated at 60 °C for one day equates to the amount of ageing that corresponds to a month stored in a 20 °C environment [21].

Biosensor ageing is a relatively well investigated field; some studies have been reported, although most remain commercial secrets [28]. The vast majority, if not all biosensor stability research is focused only on single use and shelf-life estimations, therefore, lacking the findings of "in use" ageing that is essential for novel applications such as use in semi and/or permanently integrated biosensor devices for environmental monitoring, bioprocessing and other on line or point of care use. It has been empirically noted that biosensor ageing is strongly dependent on the application and handling. Applying these differences, three scenarios were investigated in the scope of the present work: 1) well known shelf-life estimation, 2) reusability (repetitive usage and storage) and 3) continuous use (online detection of ageing while the sensor is in use). All measurements were tested with both Arrhenius (exponential) and modified Schaal (linear with several measurement points); the best fitting accelerated ageing temperature model option was selected in order to find the most suitable protocol that can be applied when studying and determining the stability and usability of a biosensor over a period of time due to age.

2. Materials and methods

2.1. Chemicals and materials

All reagent used were of analytical grade unless and commercially sourced from Sigma Aldrich (Darmstadt, Germany) unless otherwise stated: phosphate buffered saline (PBS) 100 mM, pH 7.4 tablets, iron (III) chloride, glutaraldehyde, glucose oxidase (GOx) from *Aspergillus niger* VII S (195 U mg 1) (E.C. 1.1.3.4), Albumin from bovine serum(BSA), Nafion 117, hydrochloric acid (Merck Millipore, Germany), potassium ferricyanide (Baker, Holland), D(+)-glucose anhydrous (VWR, Belgium)). Reverse osmosis (RO) water was prepared using Elga system (Elga LabWater, UK). Experiments were conducted using screen printed electrodes SPE (3 mm diameter graphite working electrode, graphite counter electrodes and sliver reference electrode) purchased from ECOBIO lab (Florence, Italy), m-stat potentiostat (PalmSens, Netherlands) and a QX3 digital microscope (Digital Blue, USA).

2.2. Fabrication of glucose oxidase biosensors

Prussian blue (PB) modified screen printed electrodes (SPEs) consisting of three electrodes: working electrode [WE], counter electrode [CE] and reference electrode [RE], were taken and the WEs were carefully modified in defined steps to produce a biosensor for glucose detection. Using a modified Ricci et al. [29,38] PB modification method, potassium ferricyanide and ferric chloride were separately diluted to 0.1 M concentrations in 10 mM aqueous solution of hydrochloric acid and then mixed together in a ratio of 1:1, forming Prussian blue. 10 µL of freshly prepared mixture was deposited onto working electrodes, fully covering the WE and incubated in the darkness for 15 min, followed by rinsing with 10 mM hydrochloric acid and copious amounts of deionized water. PB electrodes were then baked in the oven for 1 h at 100 °C. After cooling down to room temperature, 2.5 µL of 1% (V/V) glutaraldehyde (diluted with deionized water from 25% stock) was then deposited onto the surface of the working electrode and left at room temperature in the darkness to dry. For the final sensor layer, a cocktail consisting of 0.1% (V/V) Nafion 117 (diluted in distilled water from 5% (V/V) stock), 0.24 IU glucose oxidase (from powder, dissolved in deionized water) and 5% (m/V) BSA (dissolved in deionized water) was prepared. 3 µL of this cocktail was then deposited onto the surface of the WE and left to dry at 8 °C at 40% relative humidity overnight.

2.3. Shelf-life determination

For determining the shelf-life of the fabricated biosensors, 45 glucose oxidase biosensors were individually vacuum packed and each set of 15 was stored at different temperatures: room temperature (controlled, stable at 22 °C), 40 °C and at 50 °C. After every 24-h period, three biosensors from each of the three different temperatures were taken, unpacked and their enzyme activity response to glucose substrate was monitored (amperometrically at 0.06 V) at 0 and 1.5 mM glucose concentrations logged at 10 Hz for 100 s – in 20 mL of PBS or 1.5 mM glucose in PBS, respectfully. Each measurement was made in triplicate. The experiment lasted 96 h.

2.4. Reusability determination

The study to determine the reusability of the glucose biosensors, individually wrapped screen printed electrodes were divided into 5 groups of 3 and stored at different temperatures (fridge at 4 °C, incubator at 20 °C, oven at 35 °C, 45 °C and at 55 °C). After every 24 h, biosensors were taken out and their responses in 20 mL of PBS at glucose concentrations of 0 and 1.5 mM were logged for 100 s (0.06 V at 10 Hz). This experiment lasted 196 h.

2.5. Continuous use stability determination

For biosensor continuous usability determination, the experimental setup was conducted in an incubator; an individual sensor was submerged in 20 mL of 1.5 mM solution of glucose in PBS. Timed measurements were logged every 5 s for 24 h or until the stabilization of the signal fell to 0.

2.6. Equations and temperature accelerated ageing models

In cases for the determination of shelf-life and reusability, the signal time data logged between the 90th and 95th second was averaged to give a single data point:

$$\bar{I}_a = \frac{\sum_{n0}^{n1} I_i}{n} \tag{1}$$

Where $\bar{I}_a$ is the mean signal, n_0 and n_1 denote the time brackets of the signal averaged (90 and 95 s), I_i a signal at a given time point and **n** the number of measurements (5 s at 10 Hz, hence 50 measurements). As each measurement was repeated three times ($\bar{I}_a$, $\bar{I}_b$ and $\bar{I}_c$, respectfully), the mean of those was considered as data point, $\bar{I}$.

$$\bar{I} = \frac{\bar{I}_a + \bar{I}_b + \bar{I}_c}{3} \tag{2}$$

In this manner, two values were determined – $\bar{I}$ at 0 mM and at 1.5 mM, marked $I_{x,0}$ and $I_{x,1.5}$, respectfully. $I_{x,0}$ was subtracted from $I_{x,1.5}$, yielding one signal for one sensor for one time point. However, as three sensors are tested in parallels, the standard deviation (S, Eq. (3)) is also calculated and used for plotting and further calculations.

$$S = \sqrt{\frac{\sum (I_i - \bar{I})^2}{N - 1}} \tag{3}$$

where I_i is a discrete measurement, $\bar{I}$ the average of the string of measurements and N the number of data points.

100% of the signal at time point 0 is considered the signal of a fresh, stabilized biosensor before any decay is detectable. In case of continuous use ageing experiment, linear sections of the raw data are used directly for further calculations.

Two models have been investigated – one following Arrhenius ageing model (Eq. (4a)) (exponential regression of data points – Eq.