A New Rapid Method for Measuring Skatole in Pig Plasma and Fat

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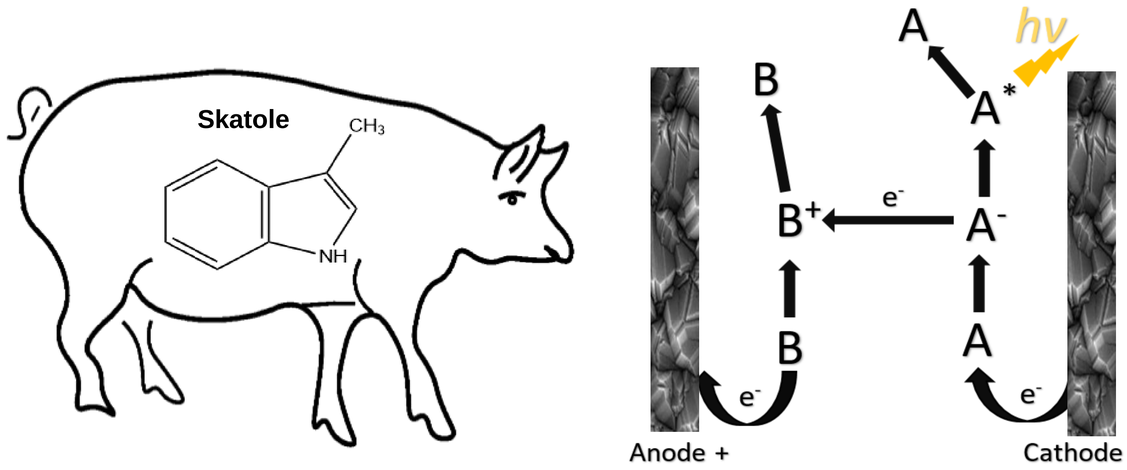
Background of boar taint, ECL and diamond electrodes



Molecular diagram of skatole (left) and principle of ECL on BDD electrodes (right)

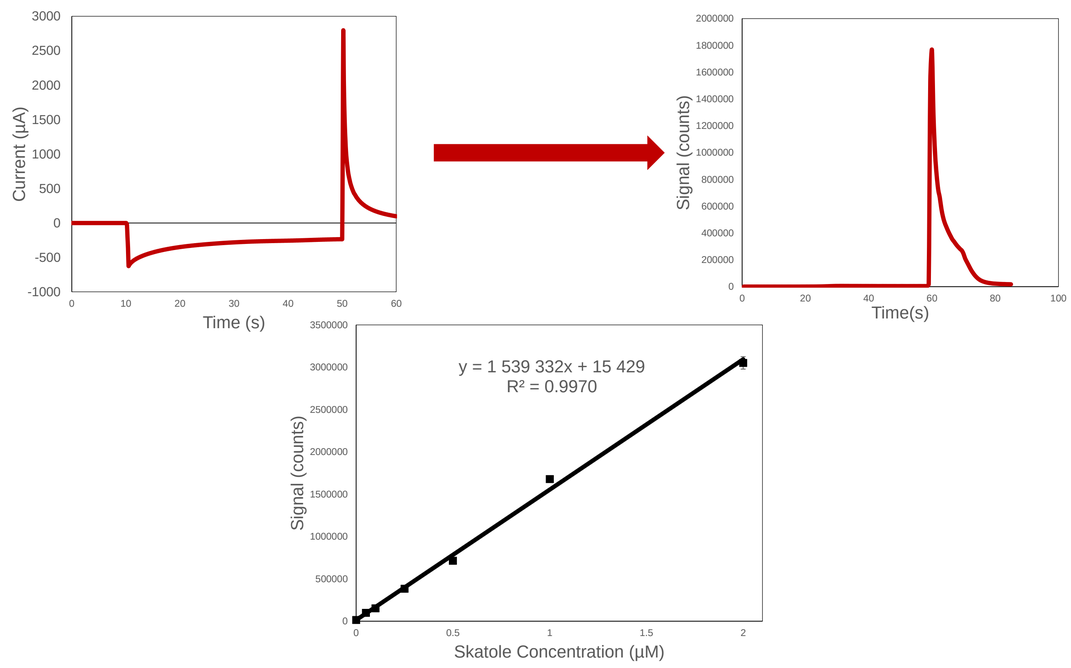
Boar Taint :

- An undesired odour found in around **10% of the meat of uncastrated adult male pigs** caused by the presence of two molecules: **androstenone and skatole**. In order to avoid the occurrence of this off-taste, many countries routinely surgically castrate male piglets without any anaesthesia.
- Recently there has been an increased desire to find alternatives (e.g **chemical detection**) to surgical castration, mainly due to concerns of the animal welfare.

Electrochemiluminescence (ECL):

- Species generated at the surface of an electrode undergo electron transfer reactions to form an excited state that then emit light.
- It inherits the merits of both electrochemical (**stability and simplicity**) and spectroscopic methods (**sensitivity**).
- **Boron-doped diamond electrodes** (BDD) provide several advantages for ECL including its wide potential window, ability to generate oxygen radicals and possibility to perform *in-situ* electrode cleaning (electrochemical activation).

Development of ECL methods



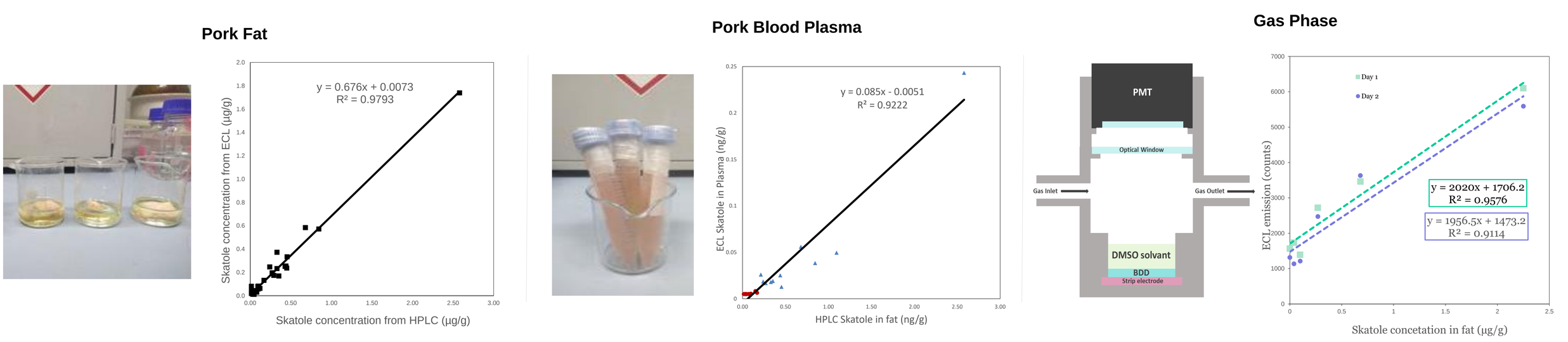
Voltage pulse in 1µM skatole solution (top left) and corresponding ECL signal (top right). Calibration graph for skatole spiked acetonitrile solution (0.05 – 2µM) (bottom).

Using a voltage pulse, **BDD electrodes** can produce an ECL signal from **skatole in acetonitrile solutions.** First a voltage of -1.8V is held to electrogenerated the superoxide radical ($O_2^{-\bullet}$). Then a voltage of 0.7V is held to oxidise skatole. The oxidised skatole and superoxide radical react to produce an excited intermediate which then relaxes, resulting in a photon emission.

BDD out performs other electrode materials, due to its ability to readily generate reactive oxygen species (ROSs) like superoxide ions.

A calibration was performed using skatole spiked acetonitrile solutions (0.05 – 2µM). giving an **unprecedented low detection limit** for a method not requiring a separation phase. (**LOD = 7.3 nM** and a **LOQ = 24.2 nM**).

Measurement of skatole in real pork tissue



Validation of ECL detection of skatole of by verification of ECL signal and known concentration of skatole. This includes liquid-liquid extraction from melted pork fat (left), double extraction from pork plasma (middle) and gas phase analysis for vaporised pork fat samples (right)

Validation of this techniques was examined by performing the **skatole ECL** in a variety of mediums:

1. **Detection in pork fat**: This was achieved through a **liquid-liquid extraction** from melted pork fat to acetonitrile. The validity of the technique was tested by comparing its ability to measure skatole content in **25 real pork fat samples** with the 'gold standard' technique of HPLC. ECL using BDD correlated well with the benchmarking technique (R^2 = 0.9793), thus showing that this method is effective in measuring the concentration of skatole in this sample type. [1-3]
2. **Detection in pork plasma**: The detection of skatole in pork blood plasma was made possible through the establishment of a **double extraction method** using rapeseed oil. By comparing the analysis of **23 pork plasma samples** with the analysis of fat samples, this method for detecting skatole was shown to be able to identify tainted meat (R^2=0.9222). By being able to perform the detection on plasma allows for testing on **live animals**. [4]
3. **Detection in the gas phase:** Detection of skatole in the gas phase (**fast sampling of pork fat**) was also demonstrated to be possible due to the high sensitivity of ECL. An analytical set-up for performing ECL gas measurements was developed, and then calibrated using a gas rig and **skatole enriched nitrogen gases**. The capability to detect skatole concentrations in a gas flow down to **3 ppm** was also demonstrated. This method was successfully tested on **6 heated pork fat samples,** (R^2 = 0.9576).

Conclusions

A method for detecting skatole using a number of sampling methods has been presented using ECL on BDD electrodes. The very high sensitive and instrumentation simplicity provides a viable route for the development of an analytical device for use in the abattoir (routine boar taint analysis) or for scientific studies. This compound, however, is only partially responsible for boar taint. To increase the interest in using ECL for this type of routine analysis, ideally a sensitive and selective method for detecting the other chemical responsible for boar taint, androstenone, should also be developed.

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[1] Method for detecting skatole in a sample of pig adipose tissue, WO2021009438A1.
[2] "Novel ECL method for the determination of skatole in porcine adipose tissue." Analytical Chemistry 94.16 (2022): 6403-6409.
[3] Procédé de détection du scatol dans un tissu adipeux de porc mâle. Patent submitted
[4] Method for detecting skatole in an aqueous solution. WO2023094753A1.