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RE: Methods for transfer a saliva based alcohol content test to a dermal patch.

Dear Ian *et. al*:

Detection and quantitation of ethanol which is highly sensitive, specific, and efficient has been a commercial target for sometime. Clearly analytical methods are useful such as gas and liquid chromatography, mass spectrometry, and NMR spectroscopy. However, those methods are best used in the laboratory and a less useful for detection and quantitation of ethanol in the field.

Enzymes have been employed for the detection and quantitation of EtOH. Enzymes are proteins that perform a particular task in a bio-catalytic way. Most of the chemistry that these enzymes do are frequently exquisitely specific in that only one alcohol reacts and only one product is produced. One enzyme molecule can catalyze the reaction of numerous substrate molecules which in itself is an amplification of the recognition signal. Alcohol dehydrogenase (ADH) and alcohol oxidase (AO) are two possible enzymatic targets for EtOH sensor development.[1] The ADH oxidizes the alcohol using a co-factor nicotinamide adenine dinucleotide. This co-factor needs to be within close proximity of the ADH. AO also oxidizes the ethanol using molecular oxygen giving rise to the production of the aldehyde and hydrogen peroxide.

Development of a patch for EtOH detection, using AO or ADH, requires what is called immobilization of the protein of interest.[2] Immobilization of enzymes make heterogeneous catalysis possible. This allows the reaction to be arrested by simple removal of the immobilized enzyme for the solution. In addition, increased stability of the protein is frequently observed due to the additional bond that takes place. There are three different methods for immobilization of AO that appears to be attractive. The first uses a polypyrrole and an acrylate derivative to entrap the protein. The full suite of kinetic parameters, changes in pH, temperature, operational stability and shelf life were determined and were adequate for the application. The second method uses an activated cellulose support that may mimic that of the solid support of the saliva test.[4] The third method uses a controlled pore glass (CPG). CPG is useful since it's a macroporous high-silica glass which can be obtained with various porosities and pore sizes. Hydroxyethyl methacrylate sorbets modified with epoxy and vinysulphate groups have been used as the co-polymer monomers. These CPG's could then be included in the patch material.

Possible Refinement of the S to P (saliva to patch); **Demonstration of technology transfer to the patch:** From the above, it evident that the enzymes that are targeted can be immobilized and these have proven to remain active in this molecular environment. The patch is most likely cellulose based. And one of methods for immobilization uses cellulose. Integration of this in the patch material would be a further avenue of discovery. Other polymers could also be integrated into the patch matrix. Dyes have been used for optical signatures of enzymatic activity with reasonable sensitivity and the ability to discern concentration (quantitation). The blue dye mentioned in our conversation could also be integrated into the immobilization process and tested for visual color changes that would be correlated to the activity of the enzyme. Which would be a signature for the presence of EtOH. Moreover, further investigations would be focused on the use of additional dyes which is designed to enhance the accuracy of the quantitation determination.

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