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A microPAD based fluorescent biosensor for ethanol detection.

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The number of home brewed or clandestinely produced and consumed alcohol in India is very high. North-east India has more than 150 tribes and each tribe has its form of local alcohol. Many locally made drinks like Sulai which is very prevalent in Assam, Rakshi made by the Miji tribe of Arunachal Pradesh are very popular. These drinks are prepared from fermented rice by distillation process and they have high shelf life. With proper standardization of these alcohols by monitoring the ethanol concentration and the preparation process, they have a good potential for commercialization. Therefore, low cost, sensitive, and portable alcohol biosensors are required.

This study demonstrates a paper microfluidic based, low cost alcohol sensor using a fluorophore molecule that changes its fluorescence upon reaction with H_2O_2 in aqueous solution. Using an enzyme catalyzed reaction that produces H_2O_2 from ethanol (equation 1), a two zone paper cut out device, containing a reaction zone and an adjacent sensor zone was developed. The ethanol containing analyte was placed in the reaction zone and a fluorescence color shift proportional to the concentration of ethanol in the analyte was observed in the sensor zone. The developed ethanol biosensor showed a limit of detection of 0.05 v/v% and the dynamic range was found to be 0.05% - 2 v/v%. Two consumer vodkas ethanol concentration was measured by this method using a paper biosensor and a smartphone camera.

$$CH_3CH_2OH + O_2 \xrightarrow{\text{(Alcohol oxidase)}} CH_3CHO + H_2O_2 \qquad \text{(Equation 1)}$$

References

Thungon, P. D., Wang, H., Vagin, S. I., Dyck, C. V., Goswami, P., Rieger, B., Meldrum, A. (2022) A fluorescent alcohol biosensor using a simple microPAD based detection scheme. *Frontiers.* (Accepted)

Thungon, P. D., Kakoti, A., Ngashangva, L., & Goswami, P. (2017). Advances in developing rapid, reliable and portable detection systems for alcohol. *Biosensors & bioelectronics*, *97*, 83–99. https://doi.org/10.1016/j.bios.2017.05.041

Nath, N., Ghosh, S., Rahaman, L., Kaipeng, D. L., & Sharma, B. K. (2019). An overview of traditional rice beer of north-east India: Ethnic preparation, challenges and prospects. *Indian Journal of Traditional Knowledge*, *18*(4), 744–757

Akyazi, T., Basabe-Desmonts, L., & Benito-Lopez, F. (2018). Review on microfluidic paper-based analytical devices towards commercialisation. *Analytica chimica acta*, *1001*, 1–17. https://doi.org/10.1016/j.aca.2017.11.010

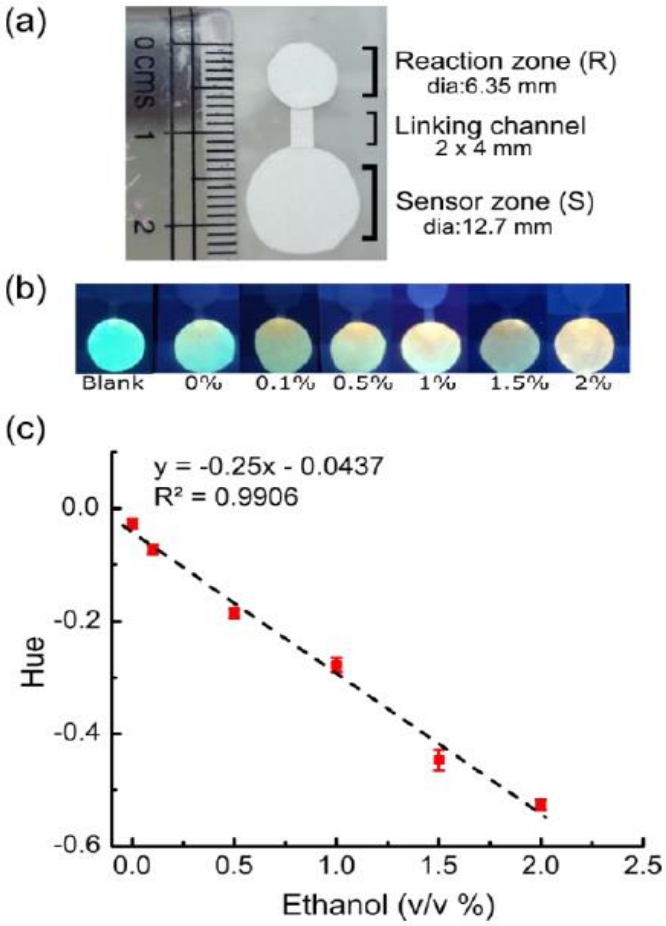

Figure 1 (a): Calibration image of the paper-based alcohol sensor; Fig (b) Photographs taken using a smartphone after the 5-minutes reaction with different concentrations of ethanol (v/v %); Fig (c) Calibration plot for the raw *hRGB* values (in radians) with a linear model fit. The error bars represent the standard deviations from three separate disks.

Table 1: Measurement of ethanol concentration in two commercial liquors. The errors represent one standard deviation from three repeats of each measurement.

SAMPLE	MEASURED V/V% (n=3)	REPORTED V/V% (n=3)
Liquor 1	43.3 ± 0.9	42.8
Liquor 2	42.5 ± 0.4	42.8