

Supplementary Information

Supplementary tables

Table S1: Pseudo code for the GUI based prediction using trained model as pickle files.

START

```
DEFINE FUNCTION 'rgb_to_hsi'(image_rgb)
  Normalize RGB values to 0-1
  Calculate Intensity as average of R, G, B
  Calculate Saturation using min(R, G, B)
  Calculate Hue using arccosine formula
  RETURN Hue, Saturation, Intensity arrays

DEFINE FUNCTION 'extract_roi_features'(image_path)
  Read image using OpenCV
  Let user select ROI (Region of Interest)
  Crop ROI from image
  Convert ROI to RGB
  Calculate mean RGB values
  Convert to HSI and extract mean Saturation
  RETURN dictionary with Green intensity and Saturation

DEFINE CLASS 'DualModelConcentrationApp'

  METHOD __init__(self, master)
    Setup GUI window
    Create radio buttons to select test type (Lead or Nitrite)
    Create a "Choose Image" button
    Create output text box for prediction result

  METHOD choose_image(self)
    Open file dialog to select an image
    Determine model path based on test type selected (Lead/Nitrite)
    TRY:
      Load pre-trained model (.pkl)
      Extract ROI features (Green and Saturation)
      Create DataFrame from extracted features
      Use model to predict concentration
      Display predicted concentration in GUI
    EXCEPT:
      Show error message in a popup

IF script is run directly:
  Initialize Tkinter root window
  Instantiate the GUI class
  Start the event loop (mainloop)

END
```

Table S2: Breakdown of cost per device

<i>Chemical/ Instrument required</i>	<i>Cost and quantity of entire purchased entity</i>	<i>Cost of one unit (INR)</i>	<i>Final cost (INR)</i>
Fixed costs			
<i>Smartphone</i>	1	10000	10000
<i>Laptop</i>	1	12000	12000

<i>Image capture box</i>	1	3000	3000
Total cost (fixed)			25000
Consumables costs			
<i>Sodium rhodizonate pellet</i>	1 (9.2 g)		~446 (entire pellet, can be reused) ~0.05 (deposited on each device)
<i>(Sodium rhodizonate)</i>	Rs.6340(25 g)	20.28 (1.2g)	
<i>Bentonite clay</i>	Rs.308(1 kg)	2 (6 g)	
<i>Graphite)</i>	Rs.10,140(1 kg)	424 (2 g)	
<i>Griess reagent</i>			
<i>(O-Phosphoric acid)</i>	Rs. 910 (500 ml)	~ >1 (~600 µl)	~ > 4
<i>Sulfanilic acid</i>	Rs. 979 (100 g)	~ >1(100 mg)	
<i>NEDA)</i>	Rs. 3528 (25 g)	~ 1.4 (10 mg)	
<i>Acetic acid</i>	Rs. 354 (500 ml)	0.14 (200 µl)	
<i>Zinc powder</i>	Rs. 890 (100 g)	0.45 (50 mg)	
<i>Whatman filter paper grade 1</i>	Rs. 1800 (100 units)	2 (1/9 th for one device)	2
Total cost for one device			~ 7

Table S3: Comparison of similar prediction models

<i>Analyte</i>	<i>Regression model</i>	<i>R-squared</i>	<i>MAE</i>	<i>RMSE</i>	<i>Cross-validated R²</i>
Lead	Linear Regression	0.87	8.29	10.64	0.81±0.058
	Ridge Regression	0.87	8.29	10.66	0.81±0.061
	Random Forest Regression	0.93	5.5	7.73	0.85±0.075
	Support Vector Regression	0.95	4.3	6.36	0.87±0.082
Nitrite	Linear Regression	0.90	5.81	8.23	0.931±0.018
	Ridge Regression	0.91	5.64	8.15	0.938±0.025
	Random Forest Regression	0.94	5.51	7.7	0.945±0.026
	Support Vector Regression	0.96	5.25	6.73	0.945±0.023

Table S4: Decision matrix showing the false negative and false positive predictions for the designed paper-based multiplexed sensor.

Decision matrix for Pb²⁺	Predicted blank for Pb²⁺	Predicted detected for Pb²⁺
Actual blank	18	2
Actual present	5	15
Decision matrix for NO₂⁻	Predicted blank for NO₂⁻	Predicted detected for NO₂⁻
Actual blank	20	0
Actual present	3	17

Supplementary figures

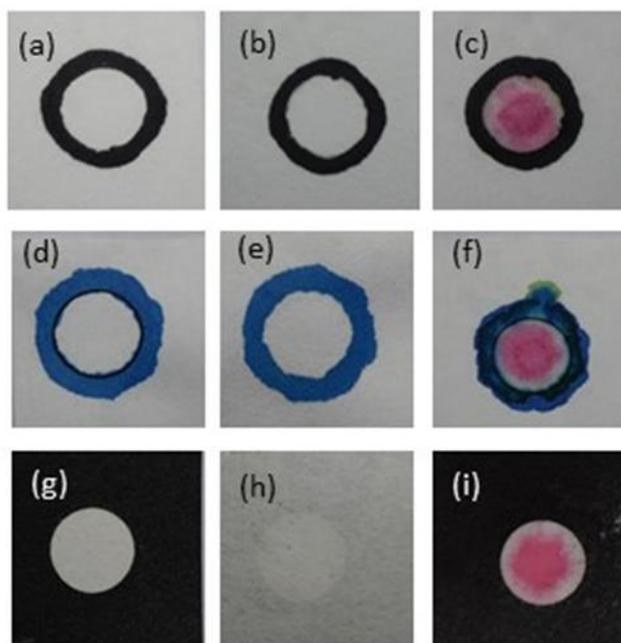


Figure S1. Effect of different fabrication mechanism of hydrophobic boundaries on paper displaying the front view, back view and view after reaction of (a),(b) and (c) permanent marker ink hydrophobic wells; (d),(e) and (f) BSA ink hydrophobic wells, and (g), (h) and (i) laser jet printed hydrophobic wells, respectively.

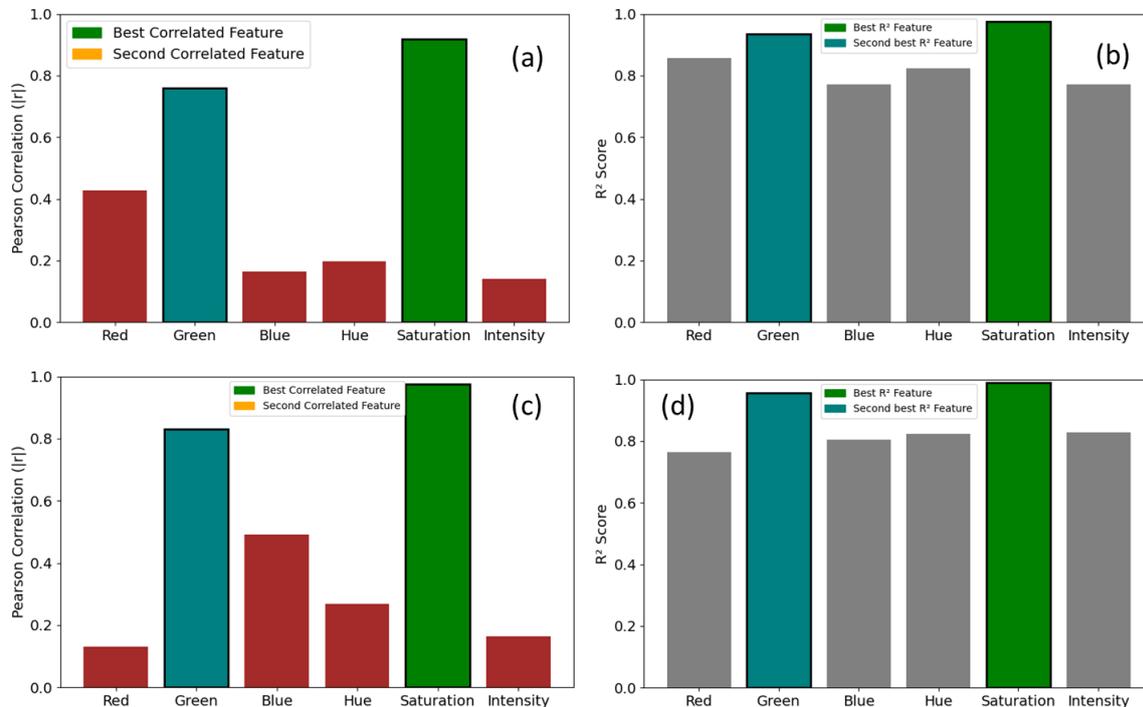


Figure S2. Selection of features for prediction of analyte concentration among RGB and HSI colour models. Comparison of the Pearson's correlation and r-squared values for Red, Green, Blue, Hue, Saturation and Intensity features for (a-b) lead and (c-d) nitrite.

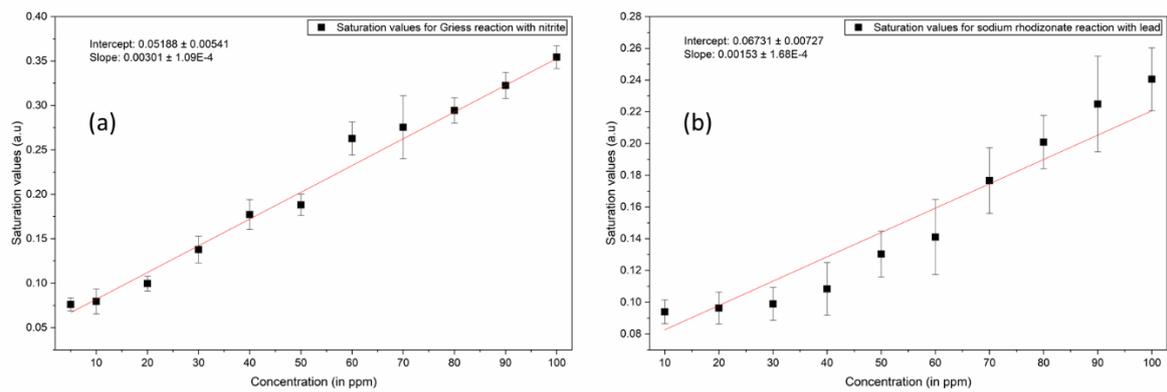


Figure S3. Linear regression for the colour developed due to (a) Griess reaction with nitrite and (b) Sodium rhodizonate reacting with lead ions with their corresponding intercept and slope values

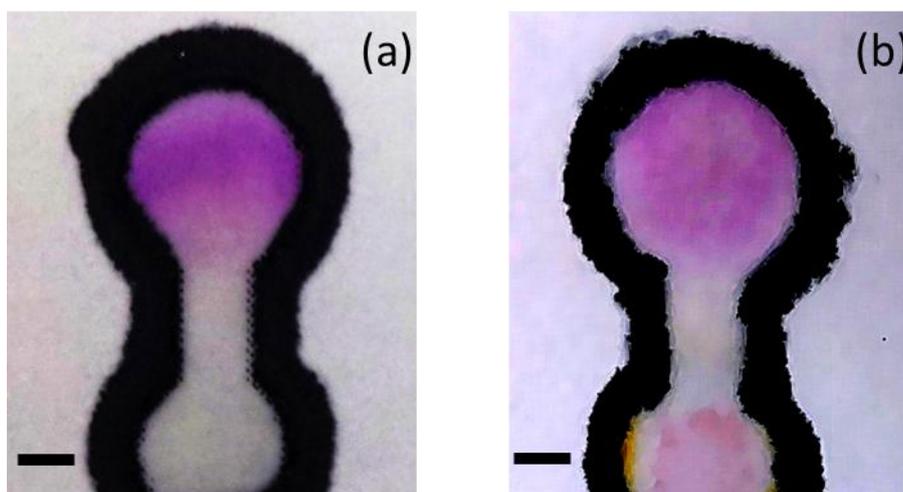


Figure S4. Comparison between developed colours due to Griess reaction on paper (a) without Chitosan coating and (b) with coating of chitosan before reagent drop-cast. (Scale bar - 1 mm)

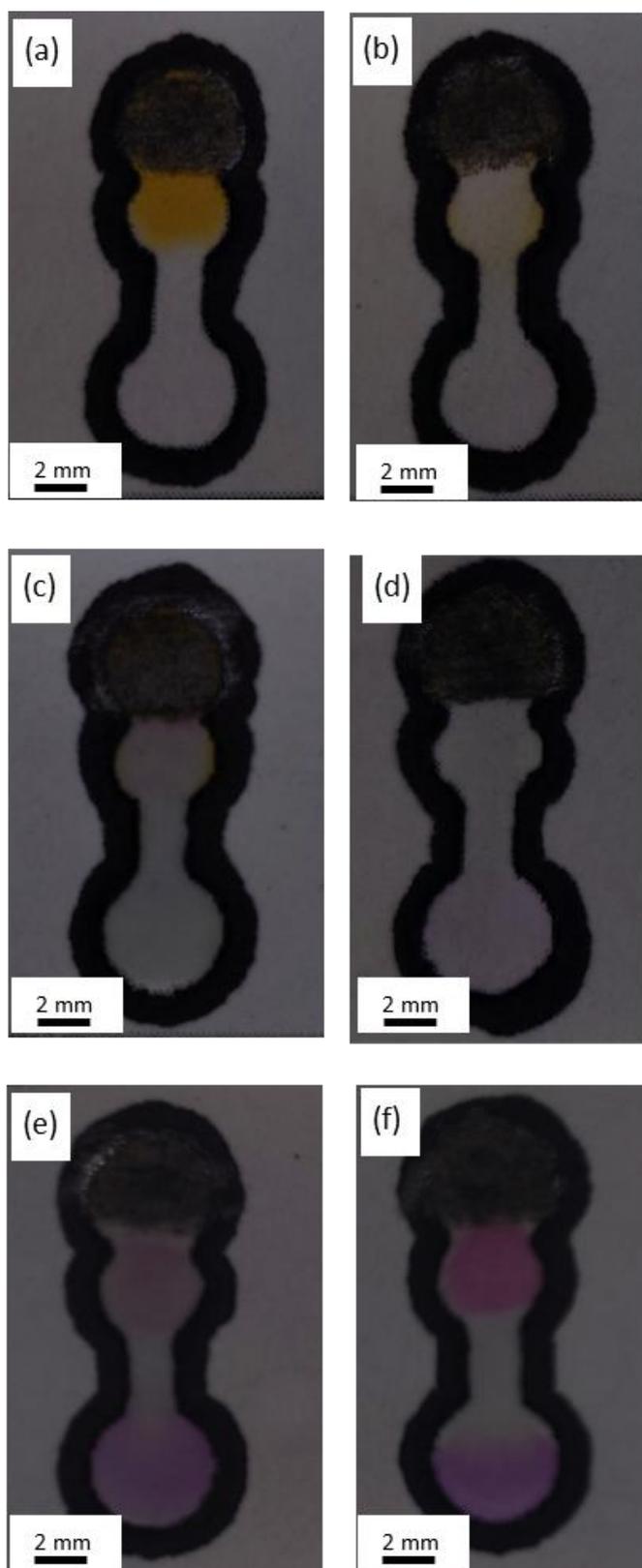


Figure S5. Representative images of the paper-based sensor under different conditions: (a) device with reagents only, showing the intrinsic yellow–orange colour of sodium rhodizonate; (b) device after addition of a blank sample, with no visible chromophore formation; (c,d) colour development observed at low analyte concentrations for lead and nitrite, respectively; (e) moderate-strength

Table S5: Mean green intensity and saturation values extracted from the regions of interest (ROIs) of the paper-based sensor images shown in Fig. S4, corresponding to blank, low-, moderate-, and high-strength analyte responses.

Samples	Lead		Nitrite	
	Green value	Saturation value	Green value	Saturation value
Sample (a) Reagent only	59.05	0.8273	74.79	0.056
Sample (b) Blank	77.14	0.0957	69.66	0.0435
Sample (c) Low lead load	55.25	0.068		
Sample (d) Low nitrite load			63.33	0.0771
Sample (e) Moderate load	47.93	0.1367	44.19	0.2238
Sample (f) High load	38.89	0.3065	42.52	0.2970

Machine Learning workflow and Implementation

1. Input features:

Green channel intensity and saturation values were used as input features, derived from smartphone capture images as given in Fig. S5, with concentration as the target for prediction using regression. The feature values derived from the images used for ML training are given in Table S5.

2. Model pipeline:

```
Pipeline([
    ("scaler", StandardScaler()),
    ("svr", SVR(kernel="rbf", C=100, epsilon=0.01, gamma="scale"))
])
```

This scikit pipeline was used for creating the ML algorithm for pre-processing and training model.

3. Training and Evaluation

Data were randomly split into training (80%) and testing (20%) sets using a fixed random seed. Model performance was evaluated using the coefficient of determination (R^2), mean absolute error (MAE), and root mean squared error (RMSE). Robustness was further assessed using 5-fold cross-validation.

4. Model execution

Trained models were serialised using Python's pickle library for subsequent deployment in the graphical user interface. The pseudo-code given in Table S1 demonstrates the working of the Graphical User Interface (GUI) for selecting Region of Interest (ROI) in the image and deriving feature values which are subsequently used to predict concentration (in ppm) and give results in terms of categories (Low strength (<10 ppm), Moderate strength (10- 50 ppm) and High Strength (> 50 ppm)) using trained model. A condition is supplied to the code to ensure no other colour than that in the pink chromophore region is used for prediction, such as:

```
PINK_HUE_MIN = 260
PINK_HUE_MAX = 340
SAT_MIN_REACTION = 0.09
```

If any other colour Hue value is picked up, the GUI shows 'Error/ Please try again'