Electronic Supplementary Material (ESI) for Analytical Methods

Supplementary information: Analytical Methods

An ultra sensitive method for rapid in-vitro catalase assay with software based approach using LabVIEW virtual instrumentation

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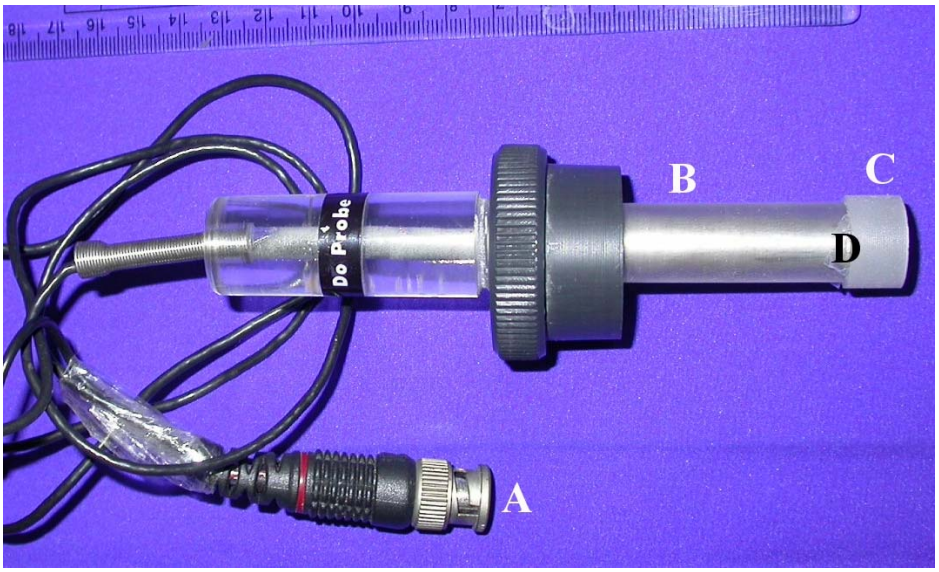
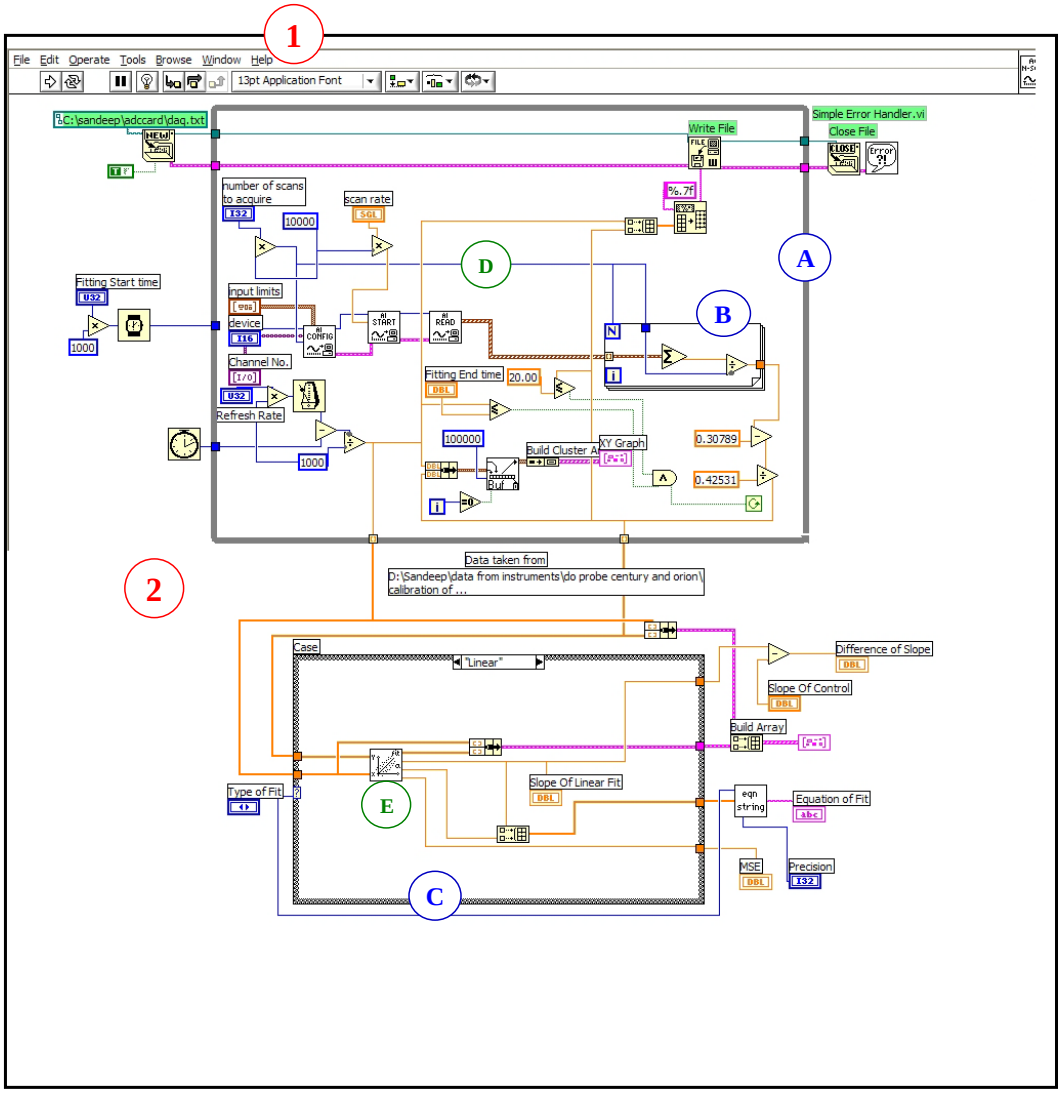
Supplementary figure S1: Dissolved oxygen probe: (A) BNC connector, (B) probe body with silver anode and gold cathode, enclosed in plastic casing, (C) O-ring and (D) Teflon membrane tied on the tip between O-ring and electrode body.

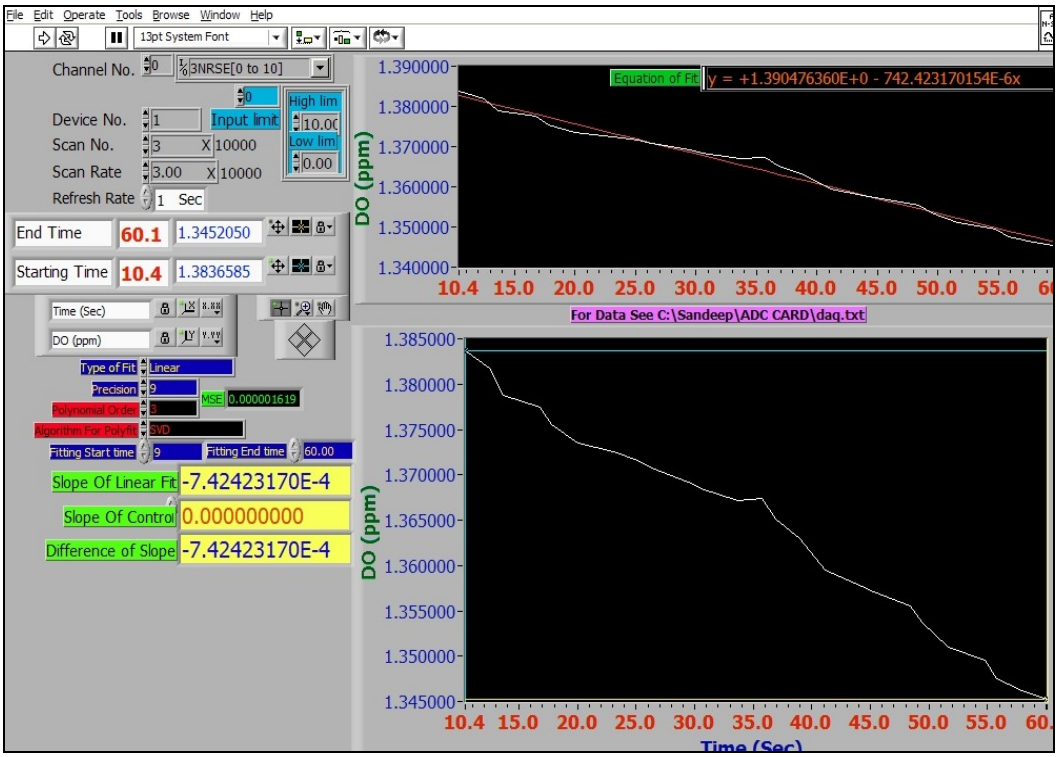


Supplementary figure S2: Block diagram and code logic used to develop LabVIEW based acquisition program to interface DO meter with computer: (1) LabVIEW menu, (2) block diagram of program, (A) while loop, (B) for loop, (C) case loop, (D) connectors and (E) individual code modules:

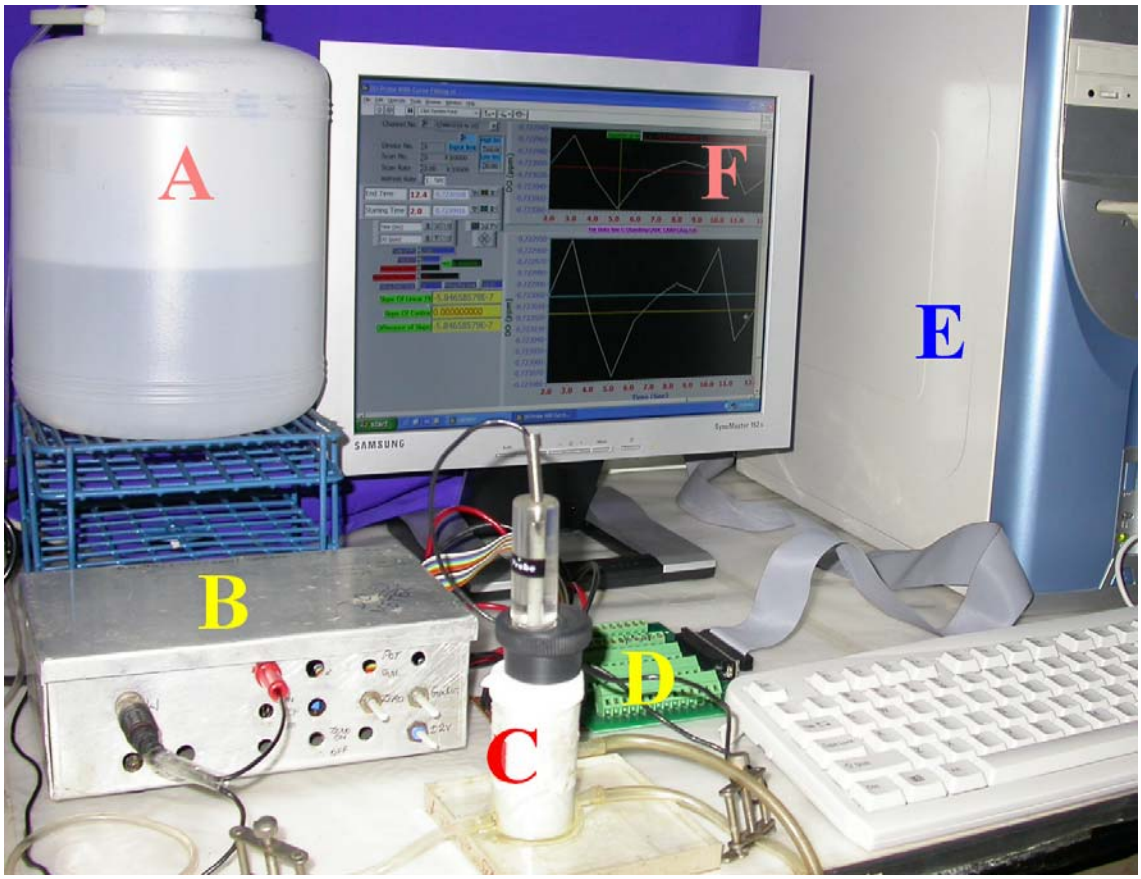


Description: An 'acquire signal' block was placed within the 'while loop' with available customisations like 'number of scans' and 'scan rate' to specify data acquisitions per second. The blocks 'input limits' was used to define operating potential range and 'channel number' for ADC channel to be used with the equipment. The 'while loop' was connected to 'continue till stopped' option to enable signal acquisition, until manually stopping the program execution.

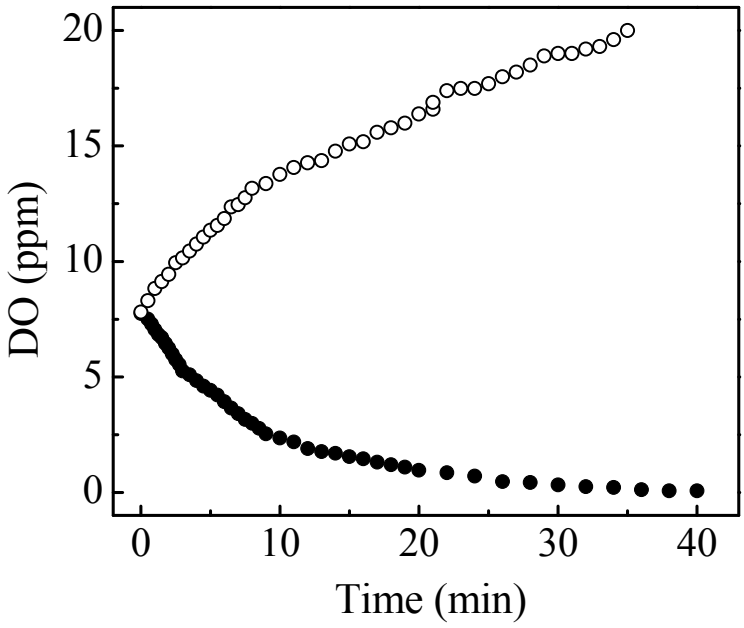
Supplementary figure S3: Guided user interface (GUI) of data acquisition program.



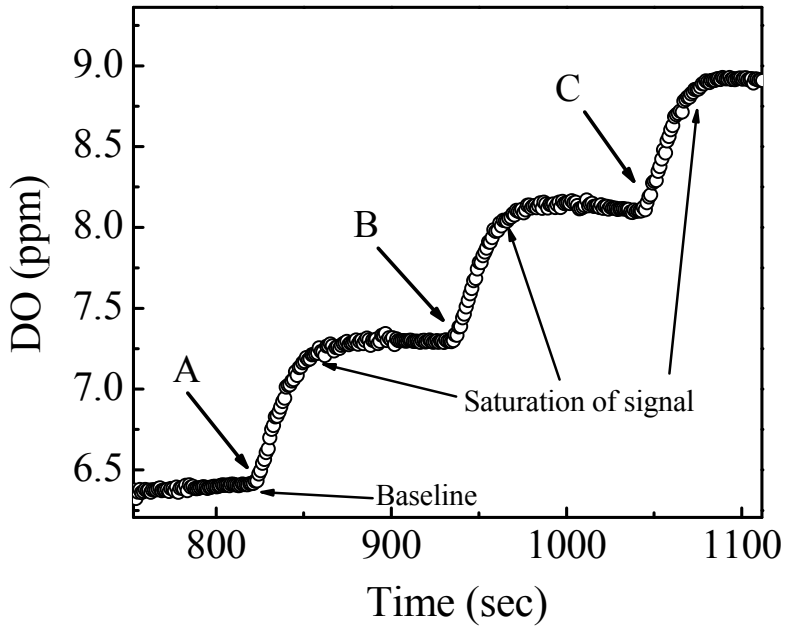
Supplementary figure S4: DO meter with all accessories: (A) buffer tank, (B) electronic circuit box, (C) flow-through reaction vessel along with DO probe, (D) I/O connector, (E) ADC card in PCI slot of computer and (F) display unit (computer monitor).



Supplementary figure S5: Changes in DO concentration in water due to purge of (●) N_2 and (○) O_2 (air) as measured using commercial DO meter.

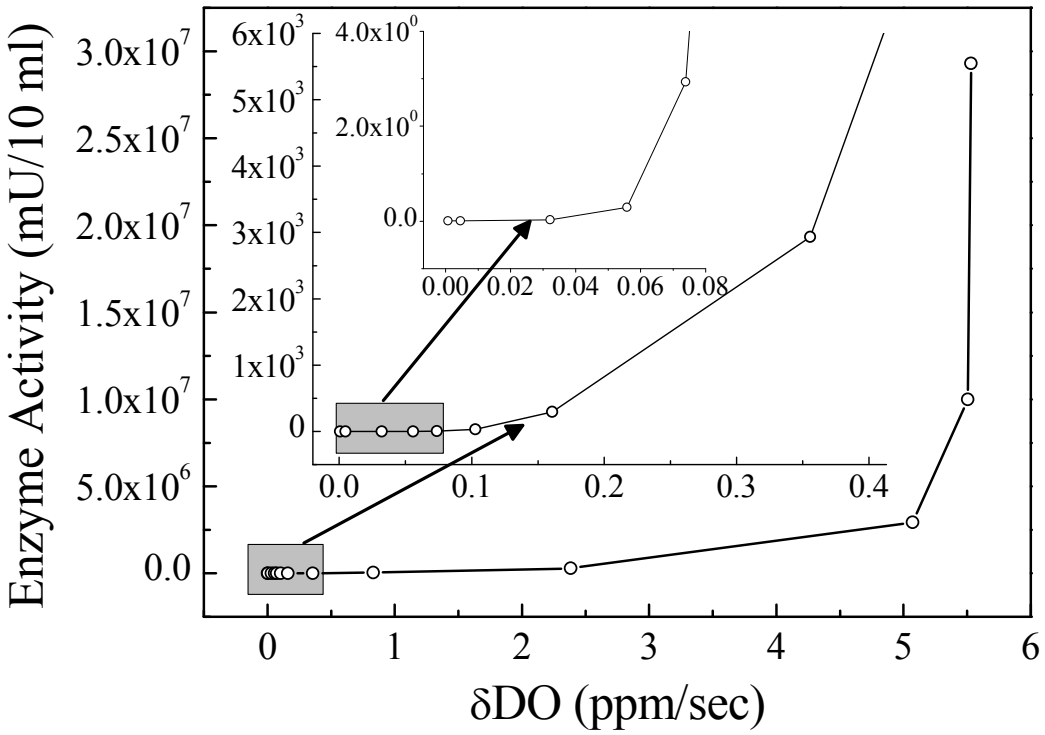


Supplementary figure S6: Response curve for assay of catalase. A, B and C are the points of addition of catalase in the medium containing fixed H_2O_2 concentration:



Description: The assay was performed using synthetic samples. At first, H_2O_2 was added in the reaction vessel and the sensor response was obtained. Once a flat baseline was reached with time, catalase solution with known enzyme activity was added in the vessel (A), which produced a steep rise in DO level till all of the H_2O_2 was consumed in the process (saturation point till B). Catalase solution was added again to the medium to initiate a further rise in DO level.

Supplementary figure S7: Standard curve for assay of catalase activity



Description: The standard curve for assay of catalase activity represented highly exponential correlation between sensor response and catalase activity. The insets in the figure represents zoomed regions for lower enzyme activities. It was quite difficult to use the equation for this exponential curve for real-time calculations using LabVIEW program. Therefore, the X and Y axis for this curve were deduced in logarithmic scale to obtain rather linear correlations between X and Y axis (see fig. 2 of main manuscript). Such modification obtained four different enzyme activity ranges for catalase which were used efficiently in LabVIEW program for calculations of enzyme activities in unknown samples. It is notable that many analytical instruments have multiple sensitivity ranges as in present case.

Calculation of theoretical resolution for the ADC card:

The theoretical resolution for the instrument was 493 ppt (parts per trillion) of DO while considering the resolution of ADC card as 152.5 μV at 0-10 V input potential range.

Given that the ADC card had 16 bit resolution, which means, in 0-10 V operation range, the ADC card had a resolution of $10V/2^{16} = 152\mu V$.

The sensitivity of the instrument was calculated as 0.30789 V / ppm of DO at signal to noise ratio in control (in plain buffer) as S/N=3, the corresponding value for 152μV shall be 493×10^{-6} ppm, which is 493 ppt.