**Supplementry Information:**

**A portable microfluidic device based Fe3O4-urease nanoprobe enhanced colorimetric sensor for the detection of heavy metals in fish tissue.**

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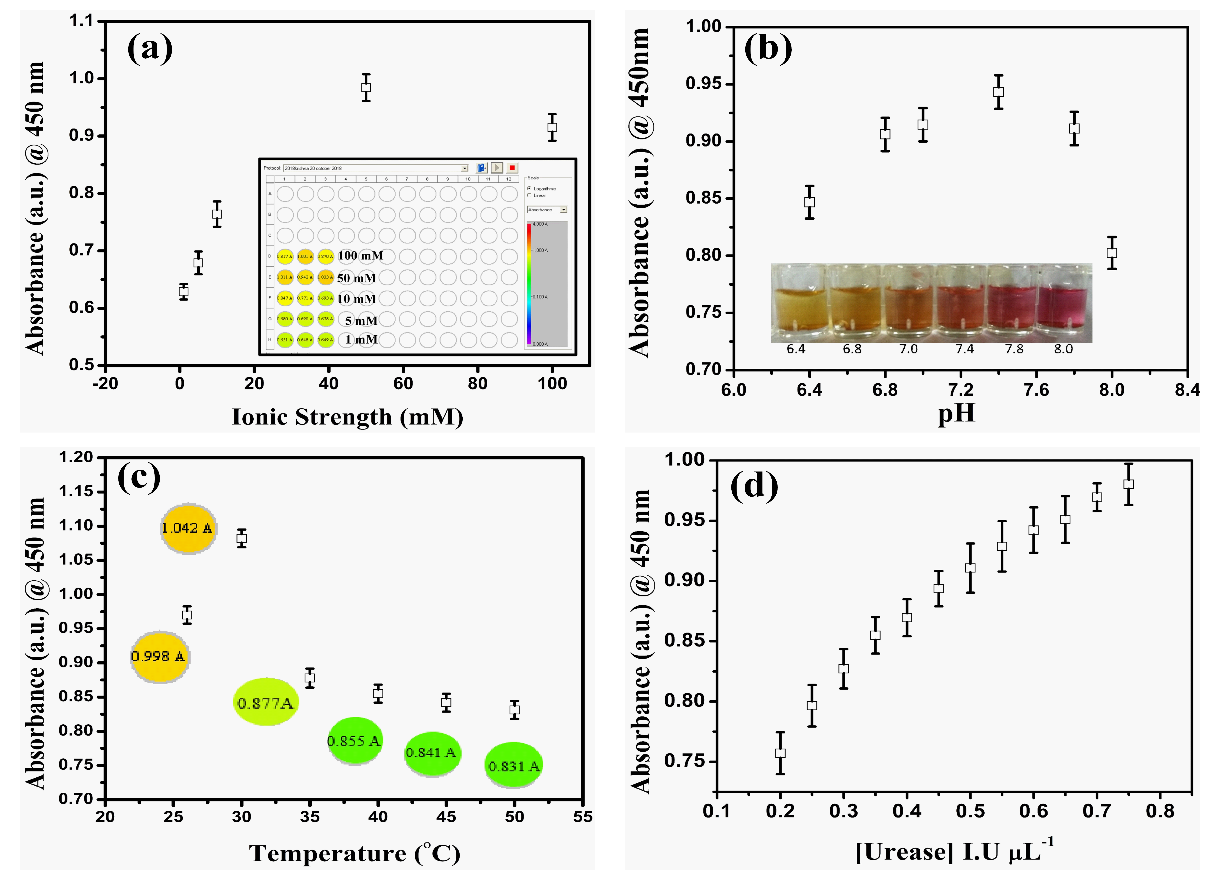
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**Supplementary Section 1:**



**Supplemetary Figure 1.** (a) Influence of ionic strength on the activity of Fe3O4MNPs-urease nanoprobe using a varying concentration of PB (1-100 mM) at 27° C (Inset: absorbance value with the color variation of individual concentration).(b) Influence of pH on the activity of Fe3O4MNPs-urease nanoprobe using the optimized concentration of PB (50 mM) at 27° C (Inset: color variation of respective pH).(c) Effect of temperature on the activity of Fe3O4MNPs-urease nanoprobe using an optimized concentration of PB (50 mM), pH 7.4 at 27° C (Inset: absorbance and color change of corresponding temperature) in triplicate format.(d) Impact of enzyme concentration on activity of Fe3O4MNPs-urease nanoprobe using optimized concentration of PB (50 mM), pH 7.4 at 27° C.

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| **Supplemetary Figure 2.** (a)Influence of ionic strength on the activity of free urease enzyme using varying concentration of PB (1- 100 mM) at 27° C in triplicate format.(b) Influence of pH on activity of free urease enzyme using optimized concetration of PB (50 mM) at 27° C in triplicate.(c) Effect of temperature on the activity of free urease enzyme using an optimized concentration of PB (50 mM), pH 7.4 at 27° C) in triplicate format. (d) Impact of enzyme concentration on activity of free urease enzyme using optimized concetration of PB (50 mM), pH 7.4 at 27° C. | |

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**Supplemetary Figure 3.** Line weaver- Burk plot obtained for activity of free urease enzyme over urea hydrolysis under optimal conditions; 0.1 mM urea in 50 mM PB (pH 7.4) at 27° C recorded at 450 nm using a multi-plate reader in photometric mode (inset shows the linearity from 0.01-100 mM of urea concentrations with Km=0.1 mM , Vmax= 0.093 and R2 of 0.959).

**Table S1:** Figures of merit for optimized assay using Fe3O4MNPs -Urease nanoprobe in 96-microwell plate format.

|  |  |
| --- | --- |
| **Parameter** | **Experimental range** |
| Urease enzyme Concentration | 0.6 I.U. /assay |
| Substrate (Urea) | 0.1 mM |
| pH | 7.4 |
| Ionic strength | 50 mM |
| Temperature | 27° C |
| Km | 0.05 mM |
| Vmax | 1.179 |
| R2 | 0.943 |

**Table S2:** The detailed comparison of the calibration curve of fish gill tissue against standard (PB) calibration curve.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Matrix** | | **Metal ions (ng L-1)** | | | **Dynamic range** |
| **Hg2+** | **Cd2+** | **Pb2+** |
| Phosphate Buffer | R2 | 0.974 | 0.920 | 0.950 | 0.5 to 50 ng L-1 |
| IC30 (ng L-1) | 1 | 5 | 0.5 |
| Sensitivity | 7.9 | 13 | 8.4 |
| Fish gill tissue | R2 | 0.971 | 0.924 | 0.959 | 0.5 to 50 ng L-1 |
| IC30 (ng L-1) | 0.5 | 1 | 0.5 |
| Sensitivity | 6.5 | 12.5 | 10.06 |