Electronic Supplementary Material (ESI) for RSC Advances.

Enzymatic electrochemical biosensor for urea with polyaniline grafted conducting hydrogel composite modified electrode

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Supplementary Materials:

Methodology:

Chemicals and electrochemical instruments

All chemicals used were of analytical reagent grades. Acrylamide, PVA, glutaraldehyde, potassium peroxodisulfate, aniline, hydrochloric acid, urea, glucose, lactose, ascorbic acid, cholesterol, sodium di-hydrogen phosphate monohydrate were purchased from Merck (Germany).Urease (jack beans) was obtained from Sigma (UK).

18.2 MΩ deionized (DI) water was used for preparation of buffer. Phosphate buffer of appropriate pH was prepared from sodium di-hydrogen phosphate monohydrate with 0.1M NaOH solution.[1]

All voltammetric and electrochemical impedance measurements were performed with an Autolab (Ecochemie B.V.The Netherlands) electrochemical analyzer (PGSTAT 12).The three electrode system consisted of modified glassy carbon working electrode (GCE, 3 mm in diameter) with platinum wire and Ag/AgCl as counter and reference electrode respectively.

Results and Discussion:

Electrochemical characterization studies using EIS

EIS was conducted to investigate and record charge transfer processes occurring at the bare-electrode–solution or modified-electrode–solution interfaces. EIS technique determines impedance of the electrode surface as a function of frequency due to variation of the interfacial properties of the interface of the electrode and solution (Rs), including electrode impedance or Warburg Impedance (Z_W) resulted from the diffusion of ions from the bulk to the electrode interface, capacitance of the electrical double layer (C_{dl}), and surface electron transfer resistance (R_{CT}). In Nyquist diagram of EIS, the semicircular part corresponds to the electron transfer limited process and its diameter represents the electron transfer resistance, R_{CT} which depends on the dielectric and insulating features at the electrode–electrolyte interface and can reveal electrode–electrolyte interface properties. Any modification of electrode surface results in change in the value of R_{CT} i.e. the resistance between the redox probe and the electrode surface. The linear extension after the semicircular part in the Nyquist diagram represents the Warburg impedance (Z_W).We characterized the immobilization process on the GCE through EIS using 1mM $[Fe(CN)_6]^{3-/4-}$ in 10 mM KCl as a redox probe in the frequency range from 0.01 to 10^5 Hz. ESI Fig. S4a† shows the Nyquist plot for the bare GCE (curve a), urease immobilized PANI (UPANI) (20 cycles of electropolymerization) GCE (curve b) and urease immobilized CPHM (UCPHM) modified GCE (curve c) respectively. The corresponding best fitted Randles equivalent circuit represented as $[R_S(R_{CT}\ CPE)Z_W]$ (ESI Fig. S4a†) where, CPE is the constant phase element.

The impedance of an AC circuit can be given by Equation 1[2]:

$Z= Z_{re}-jZ_{im}$ (1)

Where Z_{re} and Z_{im} correspond to the real and imaginary parts of the measured impedance respectively. To fit the experimental data properly, the use of CPE instead of a capacitor is needed. The CPE can be expressed as[2]:

$ZCPE = (j\acute{\omega})\alpha/C$ (2)

Where $\acute{\omega}$ denotes the radial frequency, C is the capacitance, and α is an empirical coefficient. The relationship between capacitance and impedance can be represented by the following equation [2]:

$C = -1/2\pi f^{Zim}$ (3)

Here, f corresponds to the frequency of the AC circuit (Hz). In the present system of $[Fe(CN)_6]^{3-/4-}$ medium in KCl, improved urease binding capacity of the modified electrodes could be predicted by the change in diameter of the semicircular part of the Nyquist diagram (i.e. change in the R_{CT} value). The bare electrode on the other hand, produced almost a straight line [ESI Fig. S4a†, curve a] characterising a diffusion limited phenomenon of the electrochemical process. The R_{CT} value for the bare GCE (674 Ω) was much lower than both the corresponding values obtained for the UCPHM-GCE (10.3 KΩ) [ESI Fig. S4a†, curve c] and UPANI GCE (1.58 KΩ) [ESI Fig. S4a†, curve b] due to the presence of extra layer of polymer membrane in the modified electrode.

The isoelectric point is the pH at which the total positive charges equalize the negative charges and hence the enzyme molecule remains neutral. At pH higher than the isoelectric point pH, all the acidic amino acids will have negative charge and the basic ones will remain neutral in an enzyme molecule i.e. the enzyme will be negatively charged. At pH 7 of 1 mM

$[Fe(CN)_6]^{3-/4-}$ in 10 mM KCl, urease was negatively charged (the isoelectric point being 4.5-5.2).The higher R_{CT} value in UCPHM-GCE (10.3 KΩ) than in UPANI GCE (1.58 KΩ) could be attributed to the good binding capacity of enzyme urease in the former. The electrostatic repulsive force between the redox probe of $[Fe(CN)_6]^{3-/4-}$ions and the negatively charged urease was greater in the case of CPHM-GCE surface than the PANI surface. The R_{CT} value in conducting PANI grafted PAM-PVA hydrogel membrane (CPHM) GCE (2.31KΩ) was lower than the UCPHM GCE (10.3 KΩ) [Inset of ESI Fig. S4a†, curve a, b] since there was immobilization of enzyme in the latter.

The other way of representing impedance data is the Bode Plot, where the logarithm of impedance (Z) and the phase (ø) are plotted against the logarithm of frequency (f).In the Bode plot, a horizontal line in the log Z– log|f|plot and a phase angle (ø) near 0^0 are obtained if a resistive behaviour dominates. A straight line with a slope of -1 and a ø around 90^0 signifies dominance of capacitive behaviour. Warburg impedance would be represented by a straight line with a slope of -1/2 and a ø of 45^0.[3] In ESI Fig. S4b†, curve c, with $[Fe(CN)_6]^{3-/4-}$, UCPHM GCE predicted partial capacitive behaviour (which corresponds to C_{dl}) in the frequency range of 10^2-10^3 Hz (log|f|= 2 to log | f| = 3) due to the presence of straight line with a negative slope. The Bode phase angle vs the logarithm of frequency plot indicated more capacitive nature of the GCE by UCPHM composite [ESI Fig. S4c†, curve c] than the GCE with UPANI [ESI Fig. S4c†, curve b] as well as the bare GCE [ESI Fig. S4c, curve a]. Within the applied frequency region UCPHM GCE possessed highest phase angle (29^0). Again in the low frequency region 0.1-10 Hz (log|f| = -1 to log|f|= 1),bare GCE having highest phase angle (68^0) than the UPANI GCE(45^0) and UCPHM GCE(12^0), exhibited better capacitive behaviour.

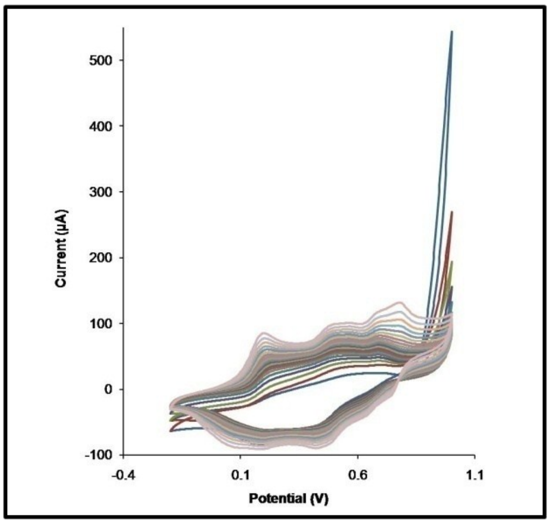

Fig. S1 Electropolymerization of polyaniline

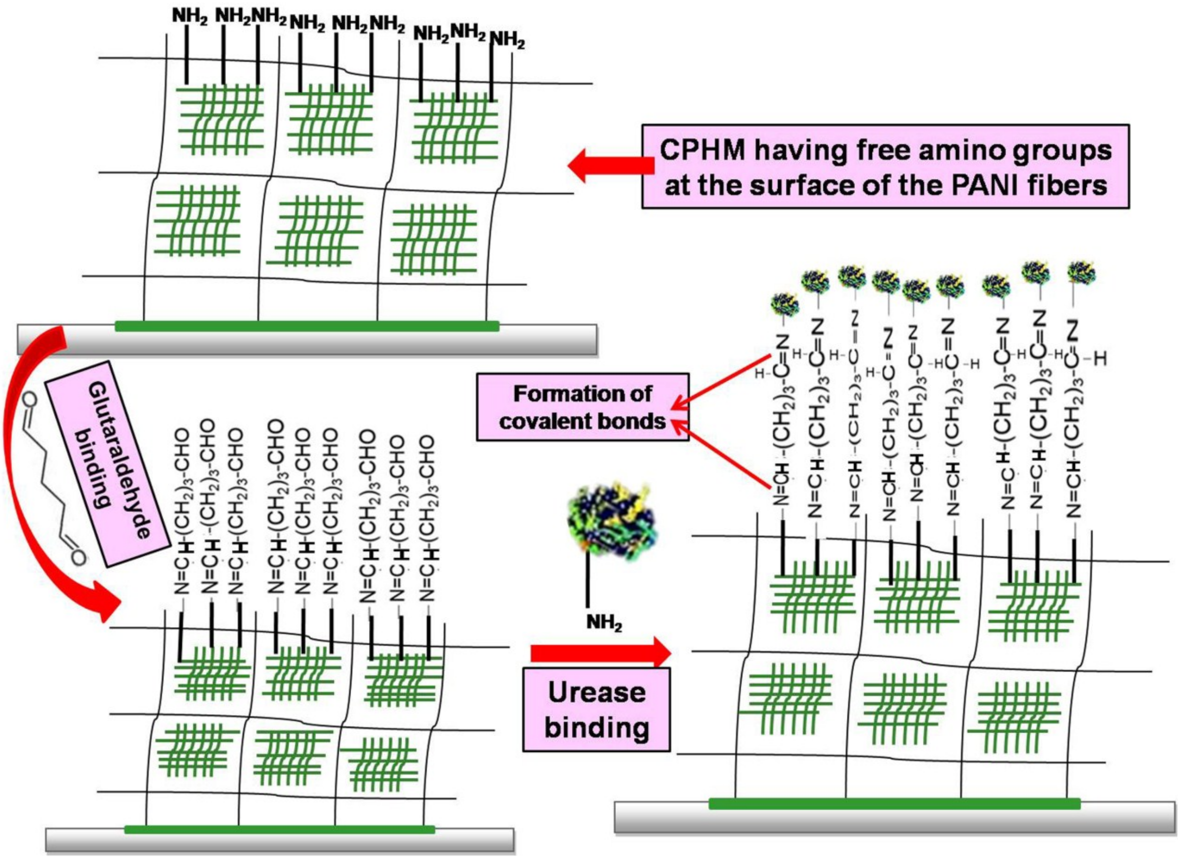

Fig. S2 Urease immobilization mechanism

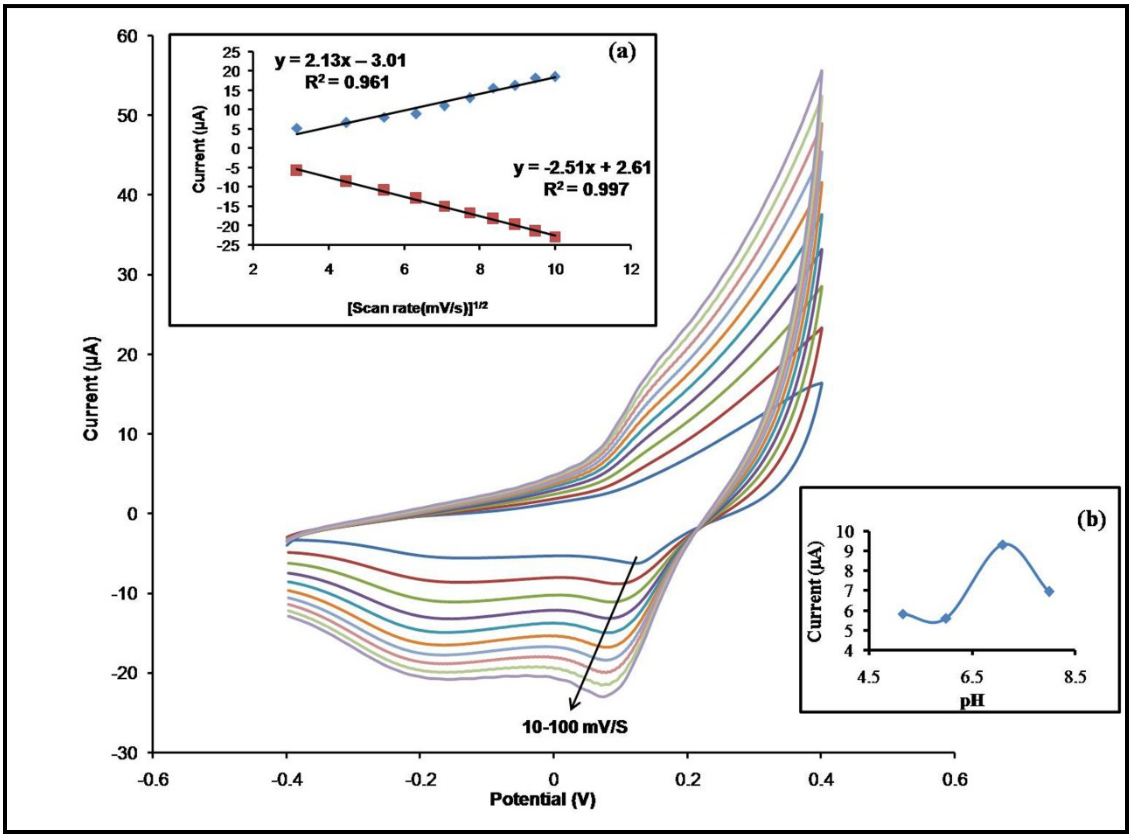

Fig. S3 CV of UCPHM GCE from 10 mV s^{-1} to 100 mV s^{-1} in 100 mM phosphate buffer, pH=7.1 containing 5 mM urea in the potential range of -0.4 to 0.4 V; the plot of (Scan rate)$^{1/2}$ vs current is shown in *Inset (a)* and the variation of current with pH of the system is shown in *Inset (b)*

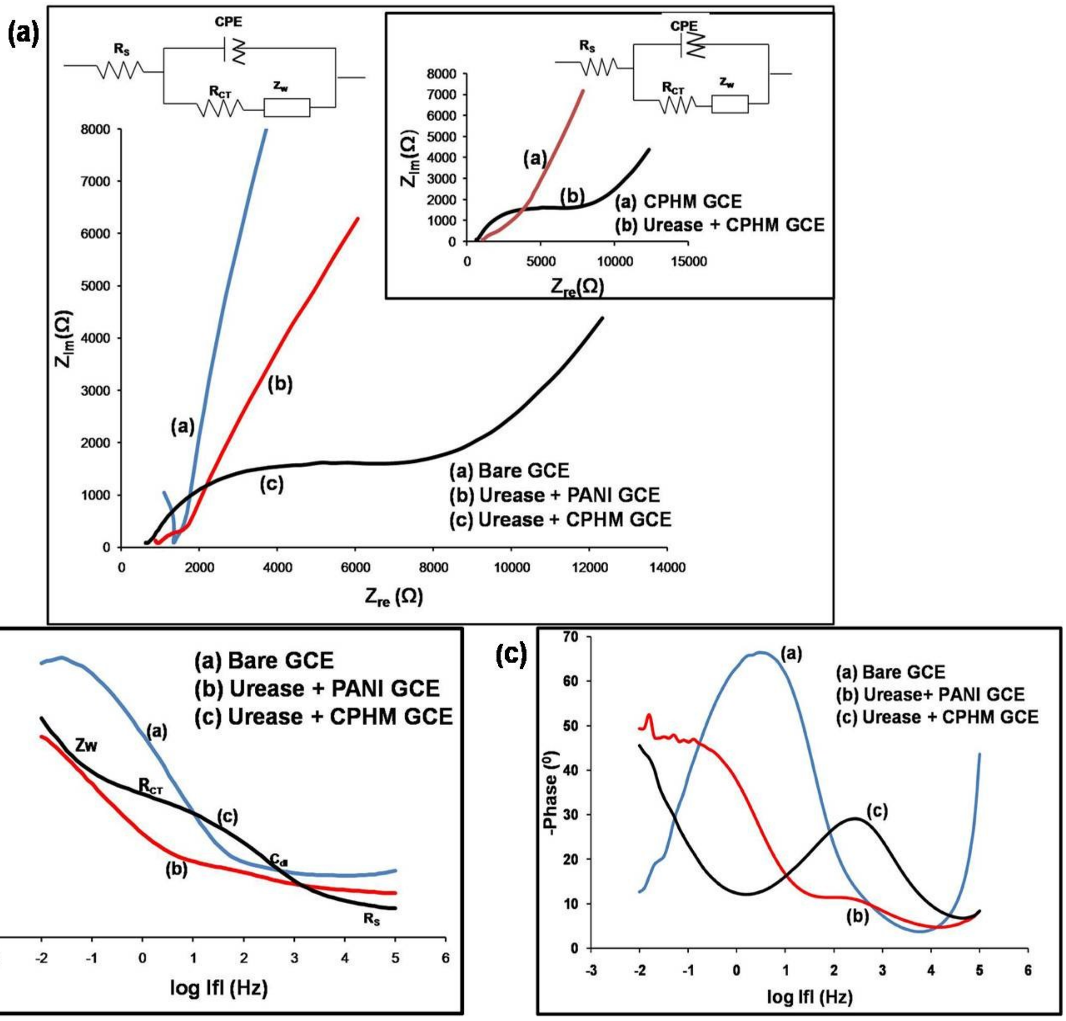

Fig. S4 (a) Nyquist plot of *(a)* Bare GCE, *(b)* UPANI GCE, *(c)* UCPHM GCE ; Nyquist plot of *(a)*CPHM GCE *(b)* UCPHM and the corresponding fitted Randles Circuit is shown in the inset, (b) Bode plot of *(a)* Bare GCE, *(b)* UPANI GCE, *(c)* UCPHM GCE, and (c) Bode phase angle diagram of *(a)* Bare GCE, *(b)* UPANI GCE, *(c)* UPHM GCE in 1mM $[Fe(CN)_6]^{3-/4-}$and 10 mM KCl in the frequency range from 0.01 to 10^5 Hz

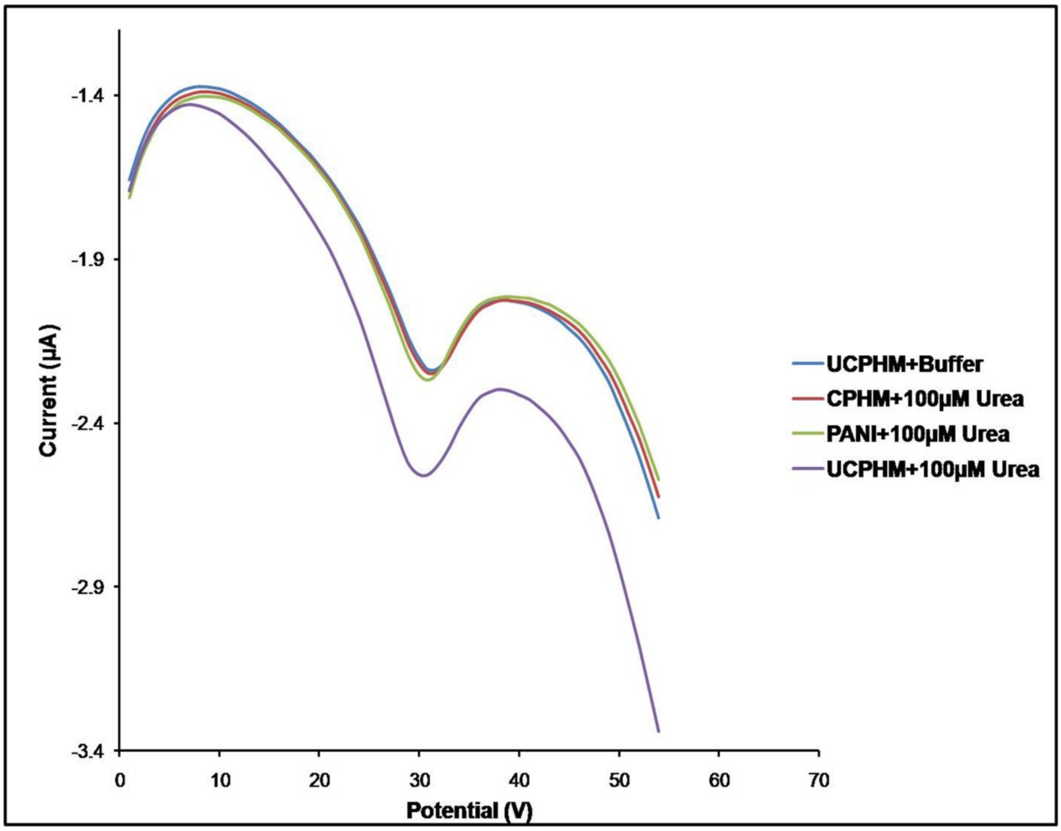

Fig. S5 Control experiment

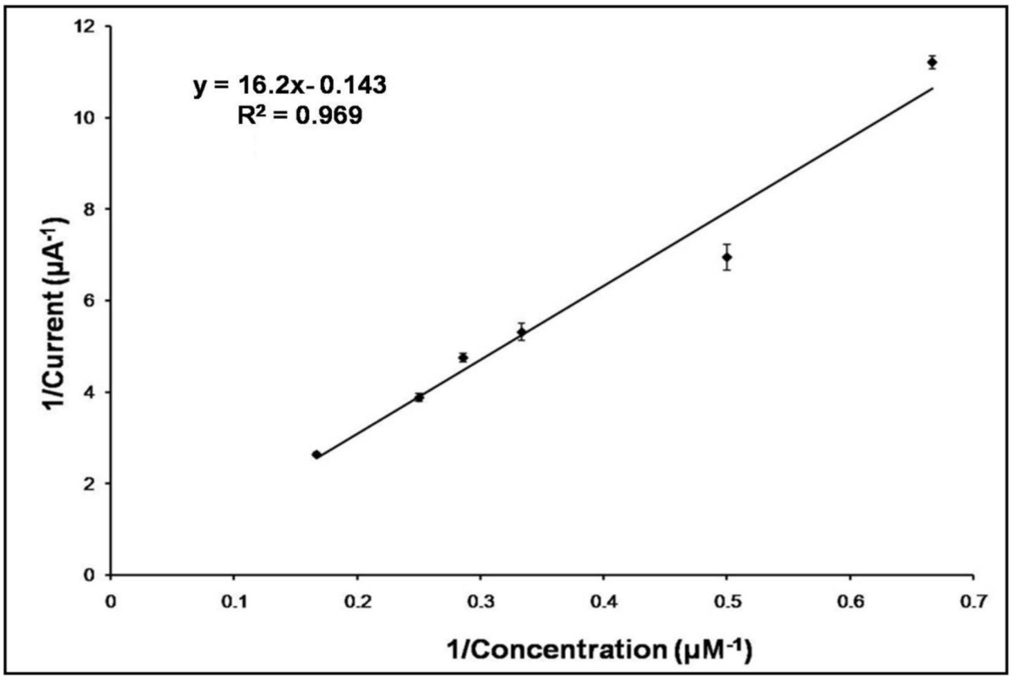

Fig. S6 Lineweaver-burk plot

500µM (within batches) (peak current-basal current)value						
	Measurement 1 (µA)	Measurement 2 (µA)	Measurement 3 (µA)	Measurement 4 (µA)	Average ±SD	%RSD
Electrode 1	1.5506	1.55	1.54	1.55	1.55±0.005	0.33
Electrode 2	1.53	1.55	1.56	1.54	1.545±0.013	0.84
Electrode 3	1.56	1.56	1.54	1.56	1.555±0.01	0.64

ESI Table1 Reproducibility Study (within batches)

ESI Table2 Reproducibility Study (between batches)

500µM (between batches) (peak current-basal current)value			
	Measurement (µA)	Average ±SD	%RSD
Electrode 1	1.55	1.55±0.005	0.32
Electrode 2	1.545		
Electrode 3	1.555		

Reference:

1. W. J. Cho and H. J. Huang, *Anal. Chem.*, 1998, **70**, 3946–3951.

2. T. Alizadeh and A. Akbari, *Biosens. Bioelectron.*, 2013, **43**, 321-327.

3. C. Sanchez, C. J. McNeil and K. Rawson, *Trend. Anal. Chem.*, 2005, **24**, 37-48.