Supplementary Information

For

L-Phenylalanine imprinted electrochemical sensor based on WS_2 nanoflowers on N,B doped graphene and its application to milk samples

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2.1. Apparatus

Field emission scanning electron microscopy (FESEM), transmission electron microscopy (TEM), x-ray diffractometer (XRD) and x-ray photoelectron spectroscopy (XPS) analysis techniques were implemented to examine the physicochemical features of the nanostructures. On the other hand, to get further insight to the electrochemical behaviors of the constructed electrodes cyclic voltammetry (CV), electrochemical empedance spectroscopy (EIS), and differential pulse voltammetry (DPV) analysis were implemented by Gamry Reference 600 potensiostat-galvanostat.

ZEISS EVO 50 SEM (Carl-Zeiss-Stiftung, Germany), and JEOL 2100 HRTEM (JEOL Ltd., Tokyo, Japan) were employed to examine the surface morphologies of nanostructures. XRD spectra of the nanomaterials were collected using a Rigaku X-ray diffractometer (MiniFlex, Japan/USA) with Cu-Kα radiation at a wavelength of 0.154 nm, and XPS analysis was performed using a PHI 5000 Versa Probe type x-ray photoelectron spectrometer (Φ ULVAC-PHI.Inc., Japan/USA). The Gamry Reference 600 workstation (Gamry, USA) was also tasked with conducting CV, EIS and DPV investigations for electrochemical characterizations of the fabricated electrodes.

2.3. GCE cleaning procedure

A mirror-like finish with fine wet emery paper (grain size 4000) was utilized for the polishing of GCE electrodes. 0.1 μm and 0.05 μm alumina slurries on micro cloth pads were successively applied to GCE electrodes. After the removal of trace alumina by ultra-pure water on electrode surface, the electrodes were sonicated in 50:50 (v/v) isopropyl alcohol and acetonitrile three times. Before the modification process, the electrodes were dried with an argon gas stream.

2.5. Sample preparation

10.0 mL of milk sample was mixed with 2.0 mL of TCA (10.0% m/v) by a vortex mixer for 40 s and centrifuged at 5000 rpm for 20 min. The supernatant was transferred to another centrifuge tube. Then, the supernatant was diluted with 0.1 M phosphate buffer (pH 7.0) for L-Phenylalanine analysis.

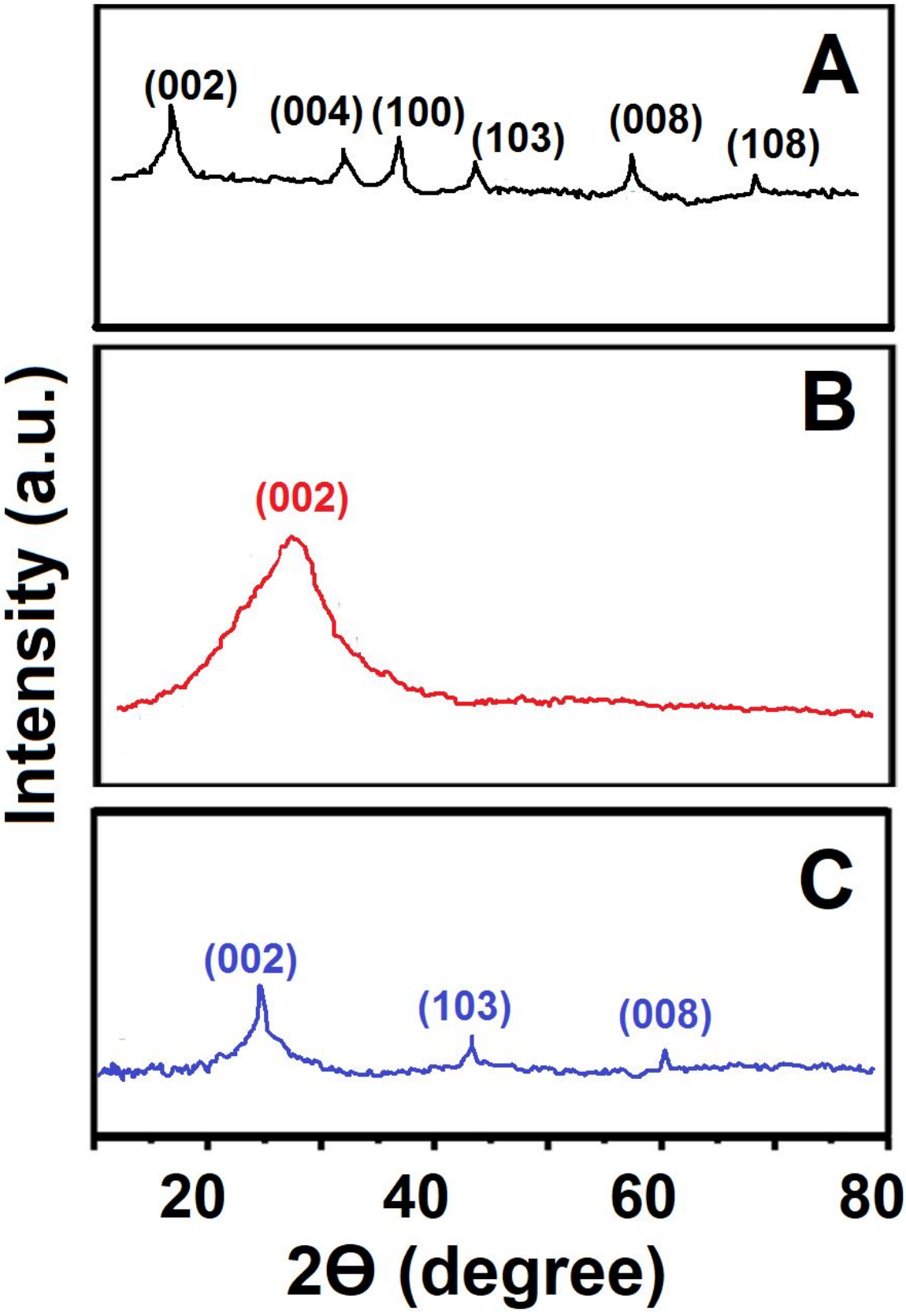

Figure S1. XRD spectra of (A) WS_2 NFs, (B) N,B-GR and (C) WS_2 NFs/N,B-GR composite

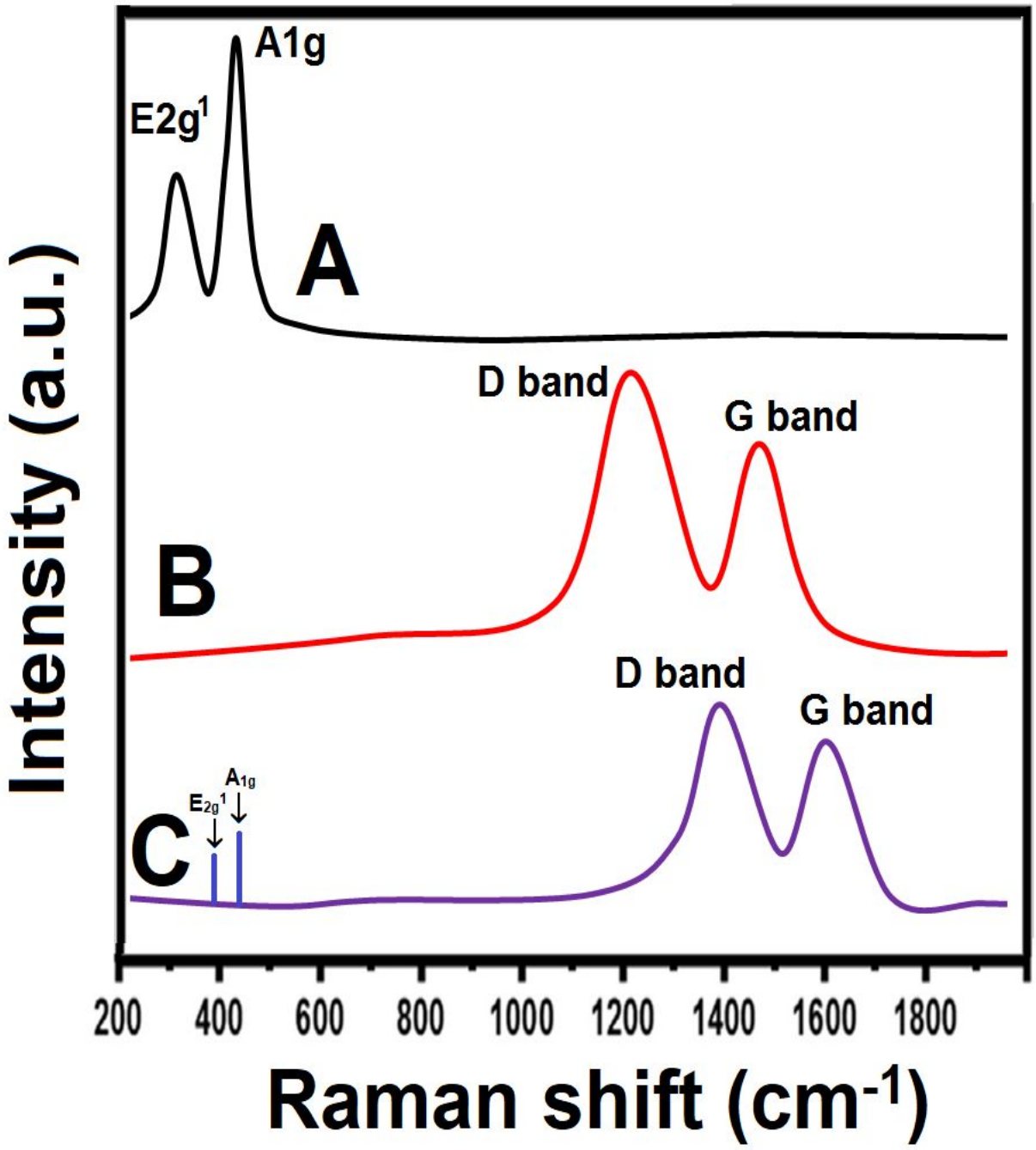

Figure S2. Raman spectra of (A) WS_2 NFs, (B) N,B-GR and (C) WS_2 NFs/N,B-GR composite

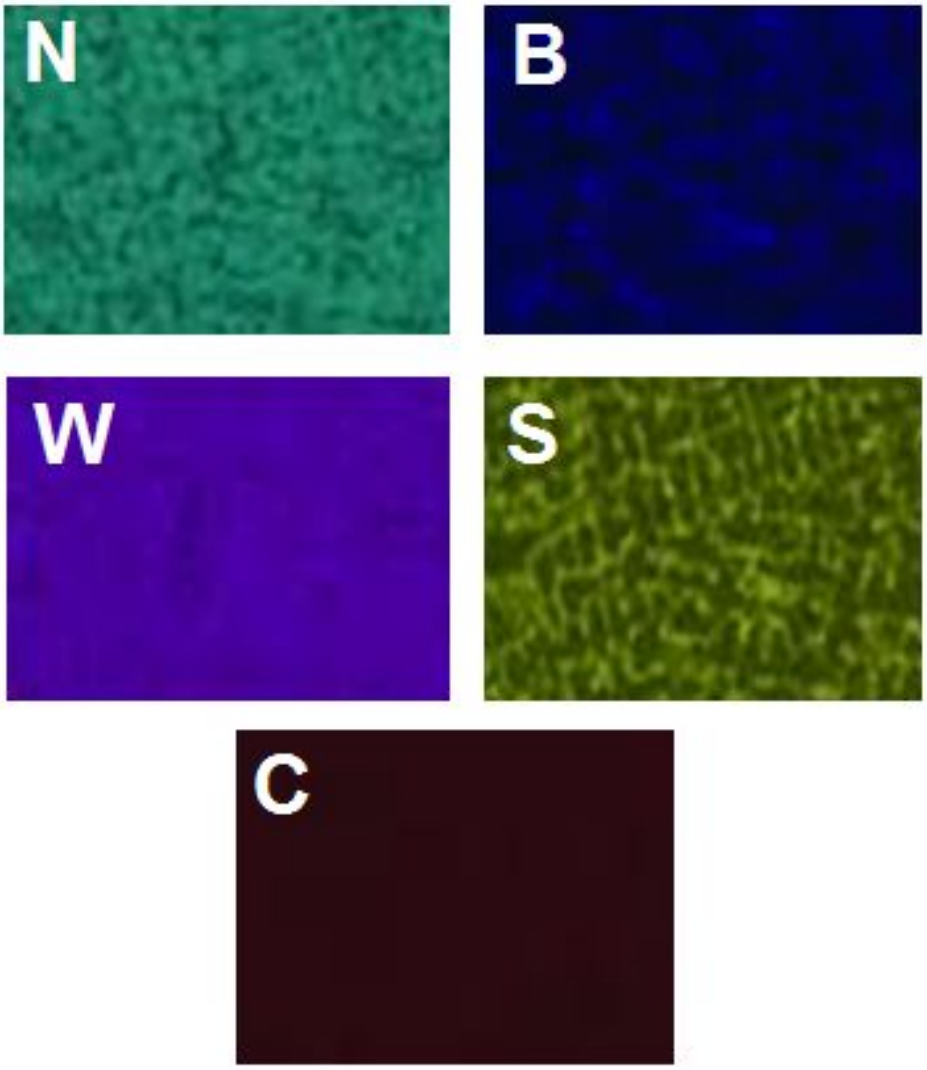

Figure S3. EDX mapping image of WS_2 NFs/N,B-GR composite

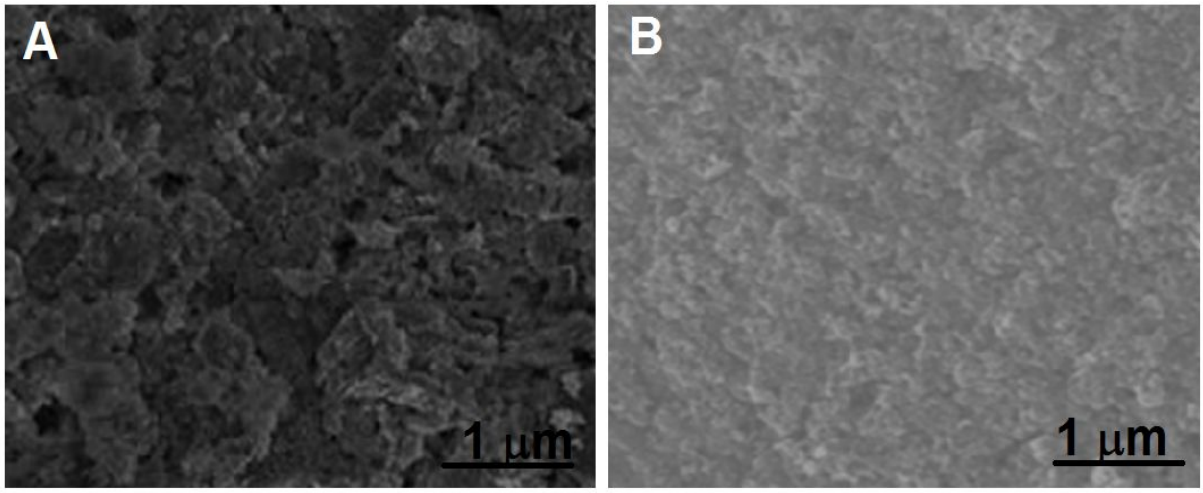

Figure S4. SEM images of (A) MIP/WS_2 NFs/N,B-GR/GCE and (B) NIP/WS_2 NFs/N,B-GR/GCE

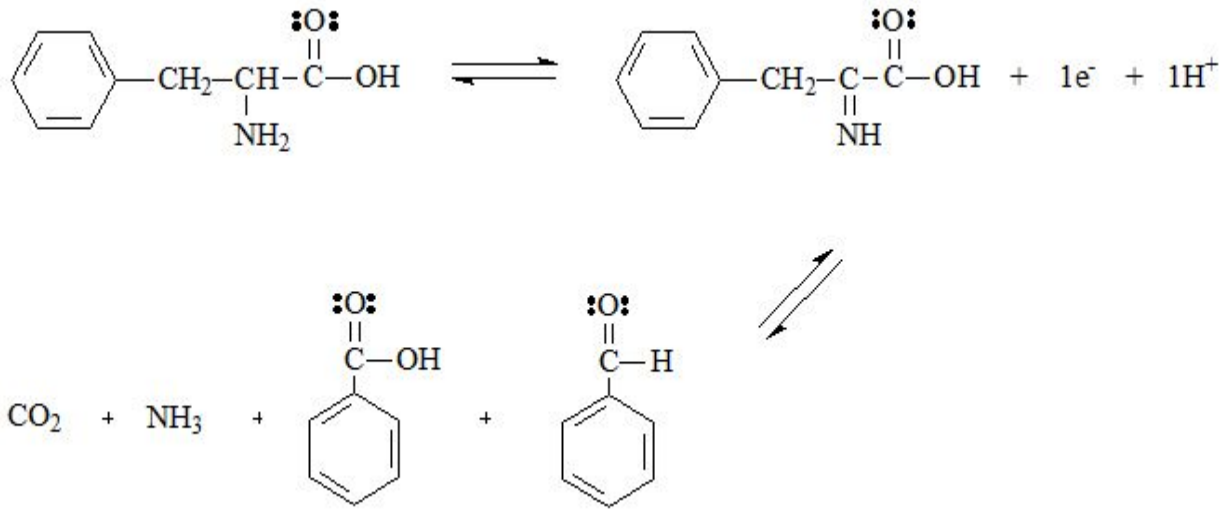

Figure S5. The electro-oxidation mechanism for PHEA on MIP/WS_2 NFs/N,B-GR/GCE

3.4. Optimization studies

3.4.1. pH effect

First of all, the effect of pH was examined (Figure S6A). pH 7.0 was chosen as the optimal pH as we consider the highest and optimal peak curves.

3.4.2. Mole ratio PHEA to Py monomer effect

Second of all, the mole ratio effect was considered in the range from (1:2) to (1:6) (Figure S6B). In accordance with Figure S6B, the current signals increased until 100.0 mM Py. These increases in current signals were caused by increased binding sites of the PHEA molecule. After 100.0 mM Py, the formation of the thicker polymer layer resulted in non-specific interactions on the electrode surface. Therefore, apparent decreases in current signals occurred and as a consequence, the optimum mole ratio (1:4) was chosen as the optimum mole ratio for the development of the PHEA imprinting electrochemical sensor.

3.4.3. Elution time effect

It is critical that the analyte molecule is completely removed from the electrode surface. When the analyte molecule is not completely removed, the desorption-rebinding kinetics of the analyte molecule may decrease, which can affect sensor sensitivity. Hence, various desorption times such as 10, 20, 30 and 40 minutes have been tried to obtain the optimum desorption time. Current signals increased up to a 20-minute desorption time, and after 20 minutes, the electrochemical signals decreased or remained constant. As a result, the optimal desorption time of 20 minutes was selected for subsequent experiments (Figure S6C).

3.4.4. Scan cycle effect

Another substantial parameter is the number of scan on sensor performance Therefore, many PHEA printed electrochemical sensors with 10, 20, 30, 40 and 50 scanning cycles have been prepared. In accordance with Figure S6D, the current signals increased up to the 20th scanning cycle due to the formation of PHEA imprinted polymer specific to the analyte molecule. In the meantime, after the 20th scanning cycle, thicker PHEA imprinted polymer formed on the WS_2 NFs/N,B-GR/GCE. Hence, the apparent decreases in current signals occurred. Eventually, as the optimum scan cycle, 20^{th} scan cycle was chosen.

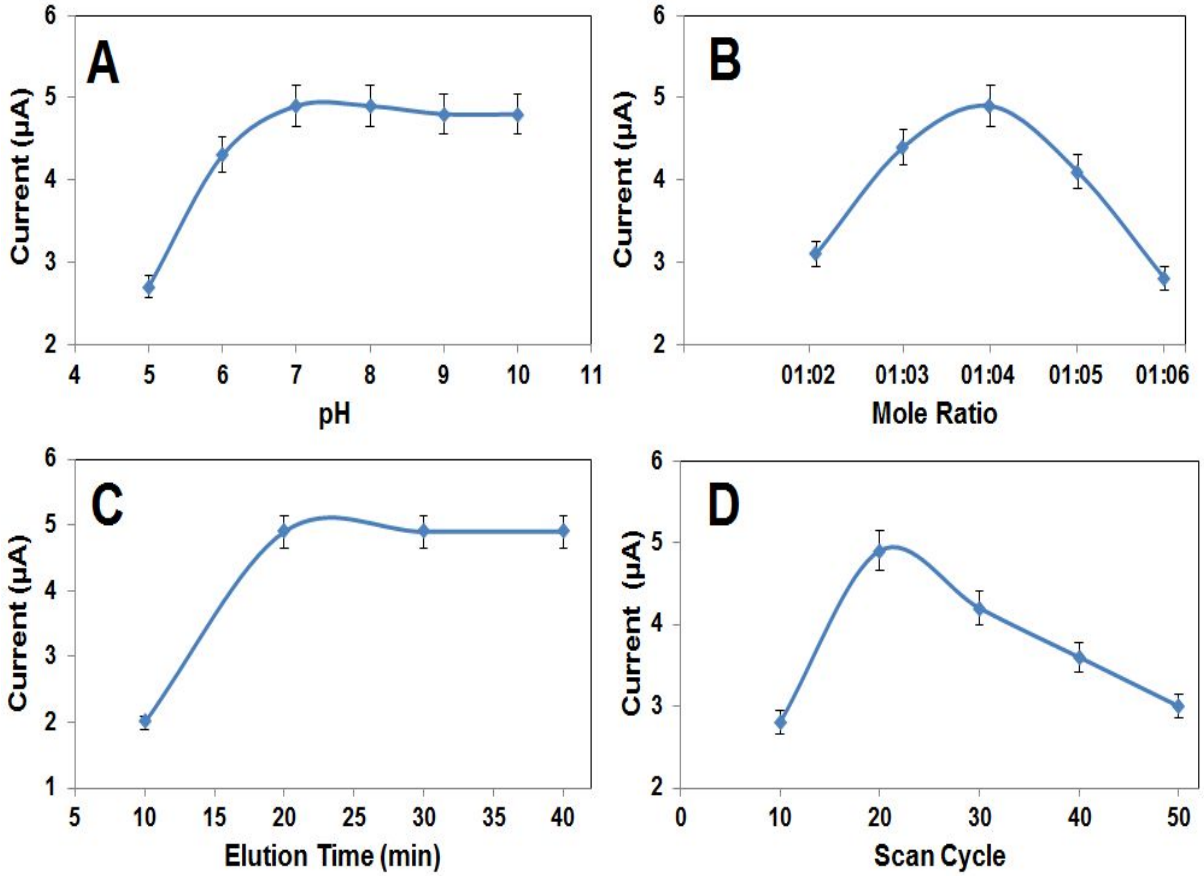

Figure S6. Effect of (A) pH, (B) mole ratio, (C) elution time, (D) scan cycle on signals of DPVs (in presence of 0.5 nM PHEA) (n = 6)

3.5. Limit of quantification (LOQ) and limit of detection (LOD)

LOQ and LOD values were computed by means of the (S1) and (S2):

$$LOQ = 10.0\, S / m \qquad \text{(S1)}$$

$$LOD = 3.3\, S / m \qquad \text{(S2)}$$

where S represents the standard deviation of the intercept, whereas m stands for the slope of the regression line.

Table S1. Recovery results of PHEA (n=6)

Sample	Added PHEA (nM)	Found PHEA (nM)	Recovery (%)
Milk	-	0.207 ± 0.004	-
	0.100	0.308 ± 0.002	100.33 ± 0.02
	0.300	0.506 ± 0.001	99.80 ± 0.01
	0.500	0.705 ± 0.003	99.72 ± 0.04

Table S2. Selectivity coefficient (k) and relative selectivity coefficient (k′) values of PHEA imprinted electrodes (MIP/WS_2 NFs/N,B-GR/GCE and NIP/WS_2 NFs/N,B-GR/GCE)

	MIP		NIP		
	ΔI (μA)	**k**	**ΔI (μA)**	**k**	**k′**
PHEA	5.00	-	0.75	-	-
DPHEA	1.00	5.00	0.50	1.50	3.33
DTRY	0.75	6.67	0.25	3.00	2.22
TYR	0.50	10.00	0.20	3.75	2.67
DOP	0.25	20.00	0.10	7.50	2.67

Analyte concentrations: 0.5 nM PHEA, 100.0 nM DPHEA, 100.0 nM DTRY, 100.0 nM TYR and 100.0 nM DOP

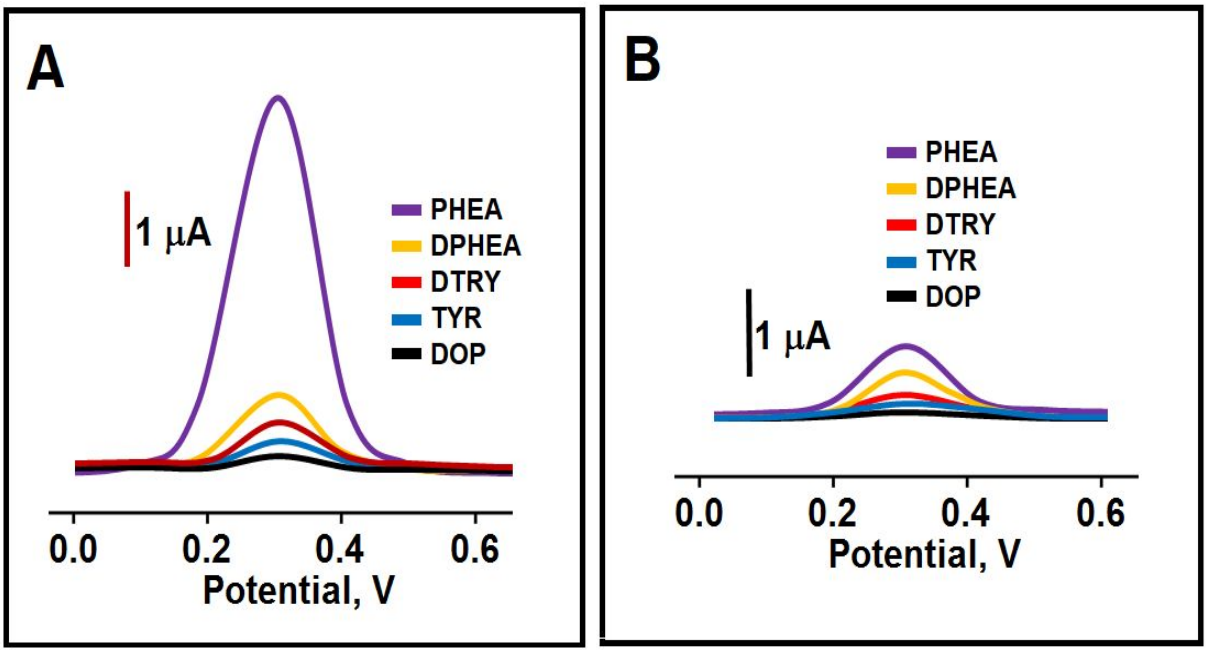

Figure S7. DPVs of (A) MIP/WS_2 NFs/N,B-GR/GCE and (B) NIP/WS_2 NFs/N,B-GR/GCE in 0.5 nM PHEA, 100.0 nM DPHEA, 100.0 nM DTRY, 100.0 nM TYR and 100.0 nM DOP

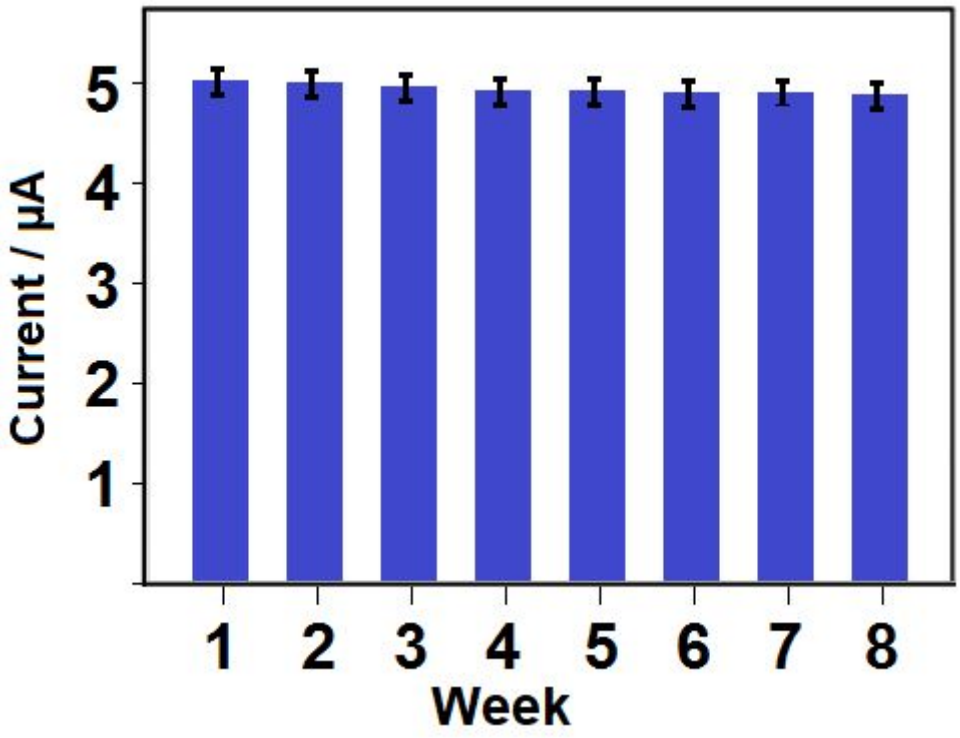

Figure S8. Stability test of MIP/WS_2 NFs/N,B-GR/GCE including 0.5 nM PHEA (n = 6) at 25.0 °C