Electronic Supplementary Material

Surface engineering of carbon microspheres with nanoceria wrapped on MWCNTs: A dual electrocatalyst for simultaneous monitoring of Molnupiravir and Paracetamol

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Experimental

Reagents and Instruments

Molnupiravir (MPV), daclatasvir (DAC), and entecavir (ENT), were kindly supplied as a gift from NODCAR, El-Dokki, Giza, Egypt. Glassy carbon spherical powder (particle size 0.4–12 micron), multi-walled carbon nanotubes (MWCNTs) (≤ 8 nm OD, 2–5 nm ID, 0.5–2 micron long, 95%), cerium(III) nitrate hydrate and paraffin oil were obtained from Alfa Aesar. Fluorolube oil was purchased from Sigma-Aldrich chemicals. To prepare stock solution, methanol was mixed with the appropriate amounts of MPV. The electrolytes Britton–Robinson buffer (BRB) , McIlvaine buffer (MB), and phosphate buffer (PB) were studied.

The synthesized CeNPs were characterized by powder X-ray diffraction (XRD), scanning electron microscopy (SEM) and high-resolution transmission electron microscopy (HRTEM, JEOL-2100F). All the electrochemical measurements [CV, adsorptive stripping square-wave voltammetry (AdS-SWV) and EIS] were performed using an EG&G PAR 384 B and an Interface 1000E Potentiostat/Galvanostat/ZRA model. A three electrode system

was used for the voltammetric simultaneous detection of MPV and PCM. The Ag/AgCl (saturated KCl) was used as a reference while platinum wire was used as a counter electrode.

Hydrothermal of synthesis of CeNPs

Hydrothermal synthetic routes have been implemented for the synthesis of CeNPs. Briefly, 5.0 g of $Ce(NO_3)_3.6H_2O$ was mixed with 60 ml of ultrapure water by stirring for 30 min. Then, about 5 mL of 0.5 M NaOH was added dropwise with constant stirring for about 0.5 h. Afterward, the mixture is transferred to a Teflon line autoclave and maintained in hydrothermal condition for 12 hours at 100 °C. Subsequently, the reaction mixture is centrifuged to separate the residue and supernatant liquid. The obtained precipitate is washed with water-ethanol several times to remove the impurities. The precipitate was then dried at 60 °C overnight and calcinated for approximately 3 hours at 400 °C to obtain CeNPs.

Preparation of real samples

The fresh serum samples were obtained from healthy volunteers at the Hospital of Assiut University, Egypt. The collected samples were centrifuged at 6000 rpm for 20 min to remove all precipitating materials. Next, 1.0 ml of each sample were diluted in 5.0 ml of the BRB solution pH 6. Next, the diluted samples were spiked with known amounts of MPV and PCM and simultaneous analysis were performed using standard addition technique.

The pharmaceutical samples (Molnupiravir-Rameda 200 mg tablets, 500 mg Panadol tablets) were supplied from a drug store. Five tablets of each were precisely measured, crushed into homogenized fine powder and dissolved in methanol followed by sonication. The solution was sonicated for 5 min in an ultrasonic bath for complete homogenization. The obtained solution was filtered using Whatman filter paper and diluted with BRB solution (pH 6.0) to achieve the required concentration for study.
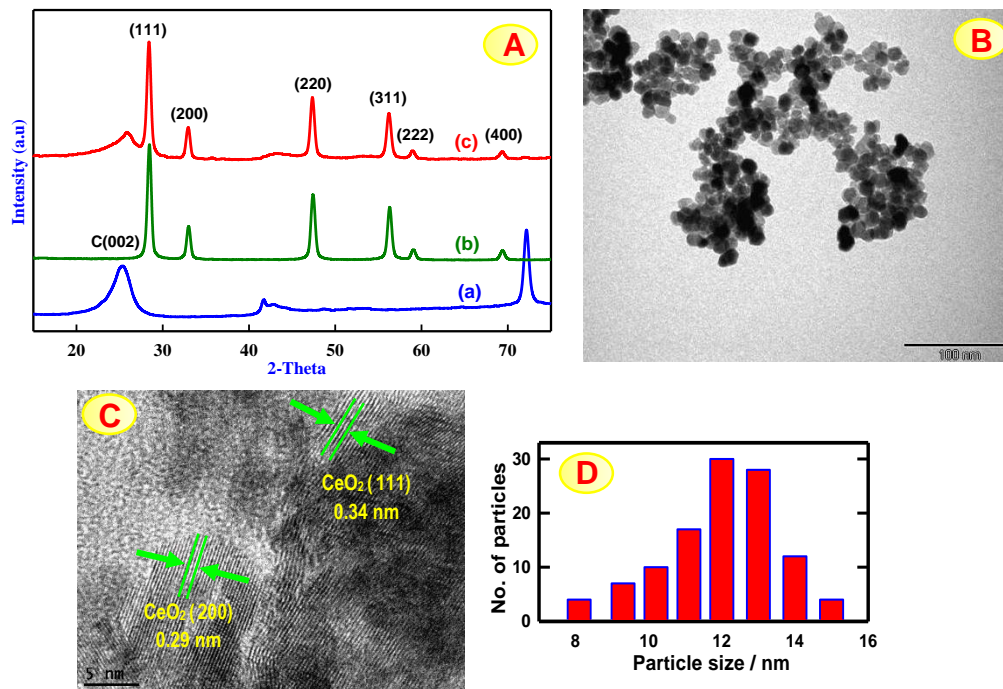
Fig. S1 (A) XRD patterns of (a) MWCNTs (b) CeNPs and CeNPs@MWCNTs. TEM (B) and HRTEM (C) images of CeNPs. Particle size distribution plot for CeNPs (D).
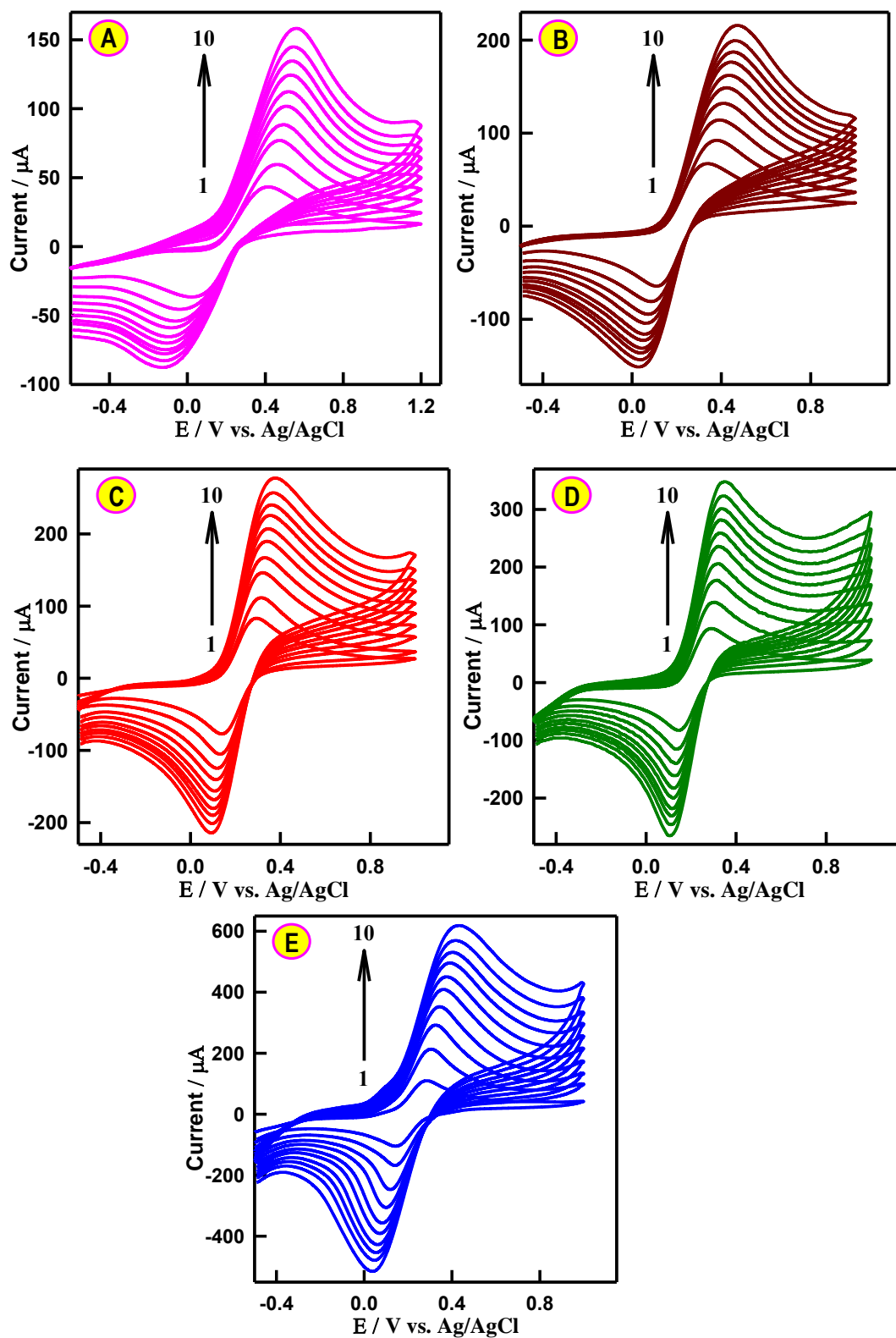
Fig.S2: CV profiles of (A) bare GCMPE, (B) GCMFE, (C) CeNPs/GCMFE, (D) MWCNTs/GCMFE and (E) CeNPs@MWCNTs /GCMFE in 5 mM $[Fe(CN)_6]^{3-/4-}$ with different scan rates (50,100,150,200,250,300,350,400,450 and 500 mVs^{-1}).
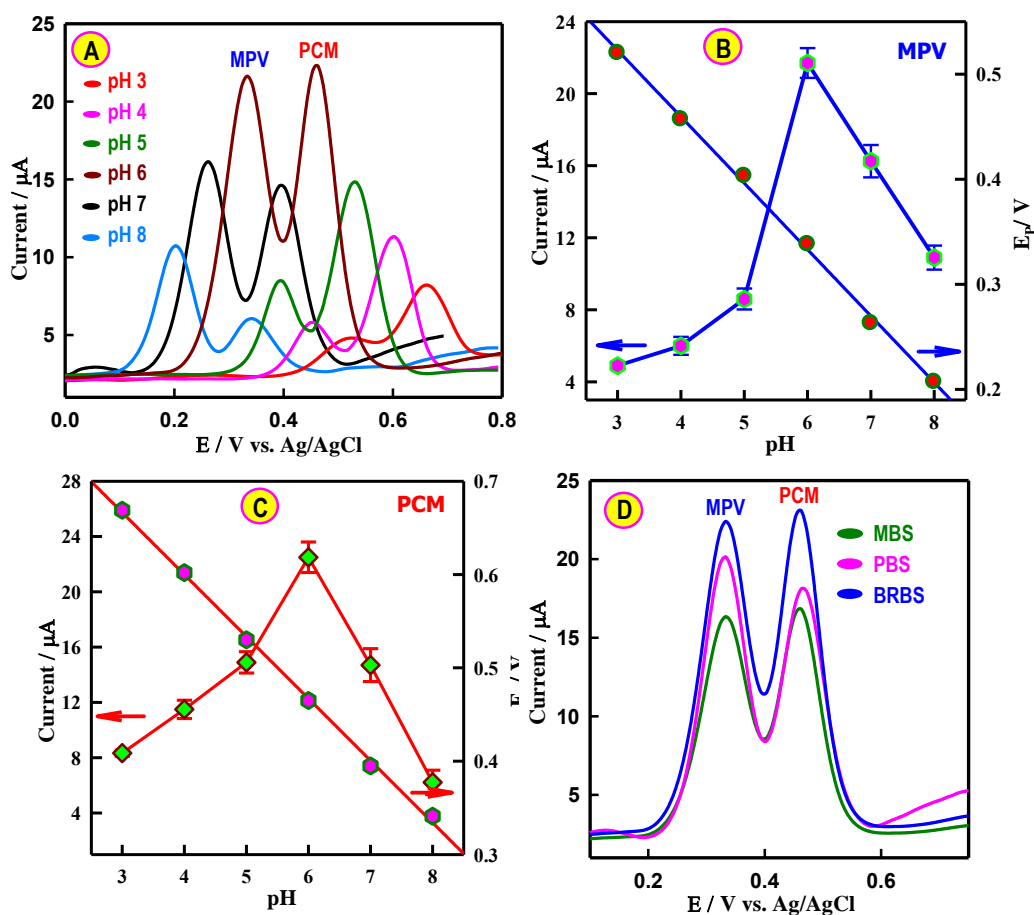
Fig. S3 (A) AdS-SW voltammograms of 2.6 µM MPV and 2.0 µM PCM on the surface of CeNPs@MWCNTs/GCMFE at different pH values (BRB solution). Effect of pH on I_P and E_P (B) MPV and (C) PCM. (D) AdS-SW voltammograms of 2.6 µM MPV and 2.0 µM PCM on the surface of CeNPs@MWCNTs/GCMFE at different supporting buffer constituents (pH = 6.0).

Optimization studies

The influence of supporting electrolyte

The supporting solution is one of the key elements affecting the behaviour of the constructed sensor. Therefore, the AdS-SWV responses of 2.6 µM MPV and 2.0 µM PCM at the CeNPs@MWCNTs/GCMFE sensor were monitored in BRB, MB and PB solutions (Fig. S3 D). Among all the supporting electrolytes, a maximum current response was monitored for MPV and PCM in BRB solution at pH 6.0.
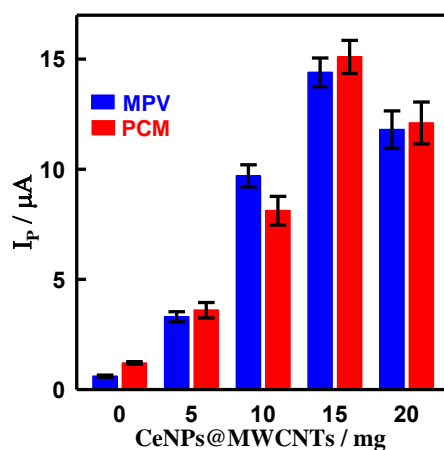
Fig. S4: Variation of MPV and PCM anodic peak currents at different weights of CeNPs@MWCNTs nanocomposite incorporated into GCMFE.

Optimization of CeNPs@MWCNTs electrocatalyst

The amount of CeNPs@MWCNTs electrocatalyst into the CeNPs@MWCNTs/GCMFE sensor composition can affect the performance of the developed sensor. Fig. S4 shows the current responses of different amounts of CeNPs@MWCNTs (5, 10, 15 and 20 mg) in BRB solution containing 2.5 µM MPV and 2.0 µM PCM . The anodic current responses increased with increments of CeNPs@MWCNTs from 5.0 mg to 15.0 mg, and decreased gradually with further increments. The larger effective surface area of the CeNPs@MWCNTs nanocomposite provided more catalytic and adsorption sites, producing a high current response. Therefore, 15.0 mg was the most suitable amount of CeNPs@MWCNTs for the construction of CeNPs@MWCNTs/GCMFE sensor to simultaneous detection of MPV and PCM.
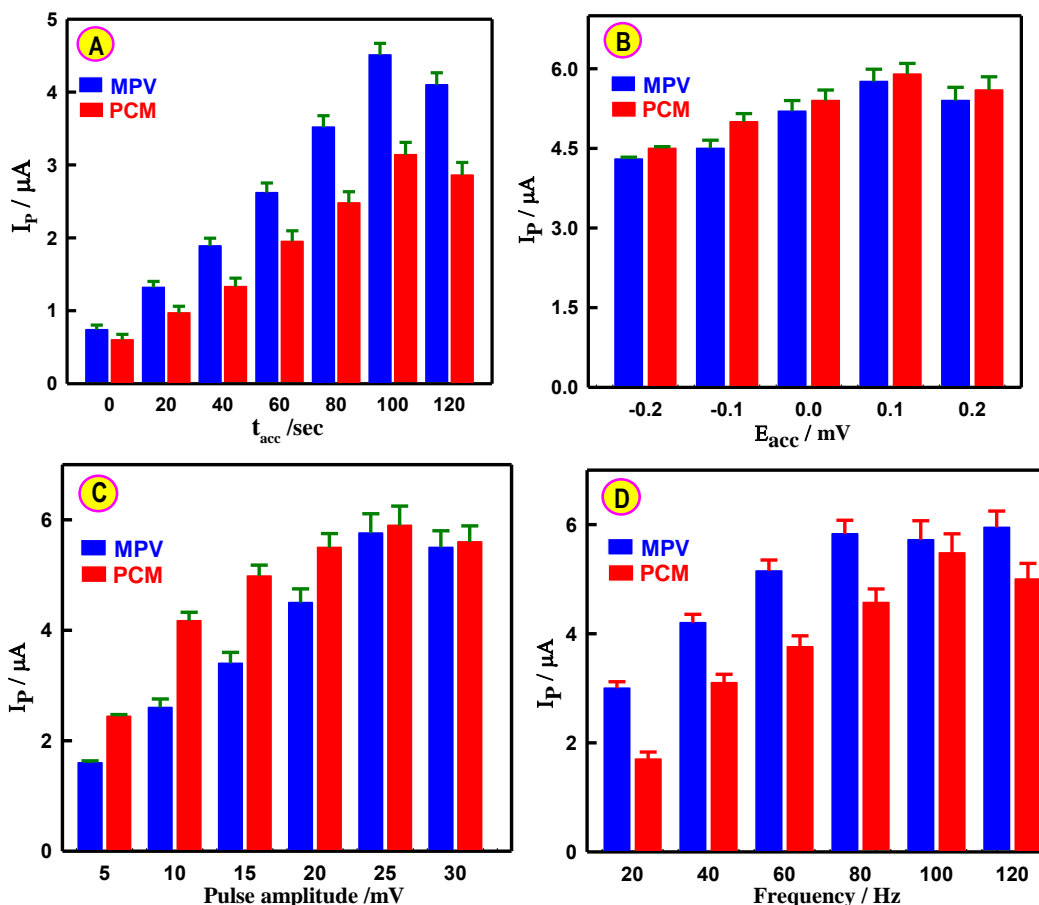
Fig. S5 Effects of (A) accumulation time and (B) accumulation potential on the peak currents of MPV and PCM. Effects of (C) pulse amplitude and (D) Frequency on the peak currents of MPV and PCM. Error bar represents the standard deviation of triple measurements.

Optimization of AdS-SWV parameters

AdS-SWV parameters such as accumulation time, accumulation potential, pulse amplitude and frequency influence the current response and the shape of AdS-SW voltammograms. Therefore, the effect of these parameters on the current responses of MPV and PCM in BRB solution of pH 6 was studied. To obtain low background current, sharp AdS-SWV peaks and high peak current response for the simultaneous determination of MPV and PCM, the optimized values are accumulation time (100 sec), accumulation potential (+0.1 V), pulse amplitude (25 mV) and a frequency of 100 Hz.
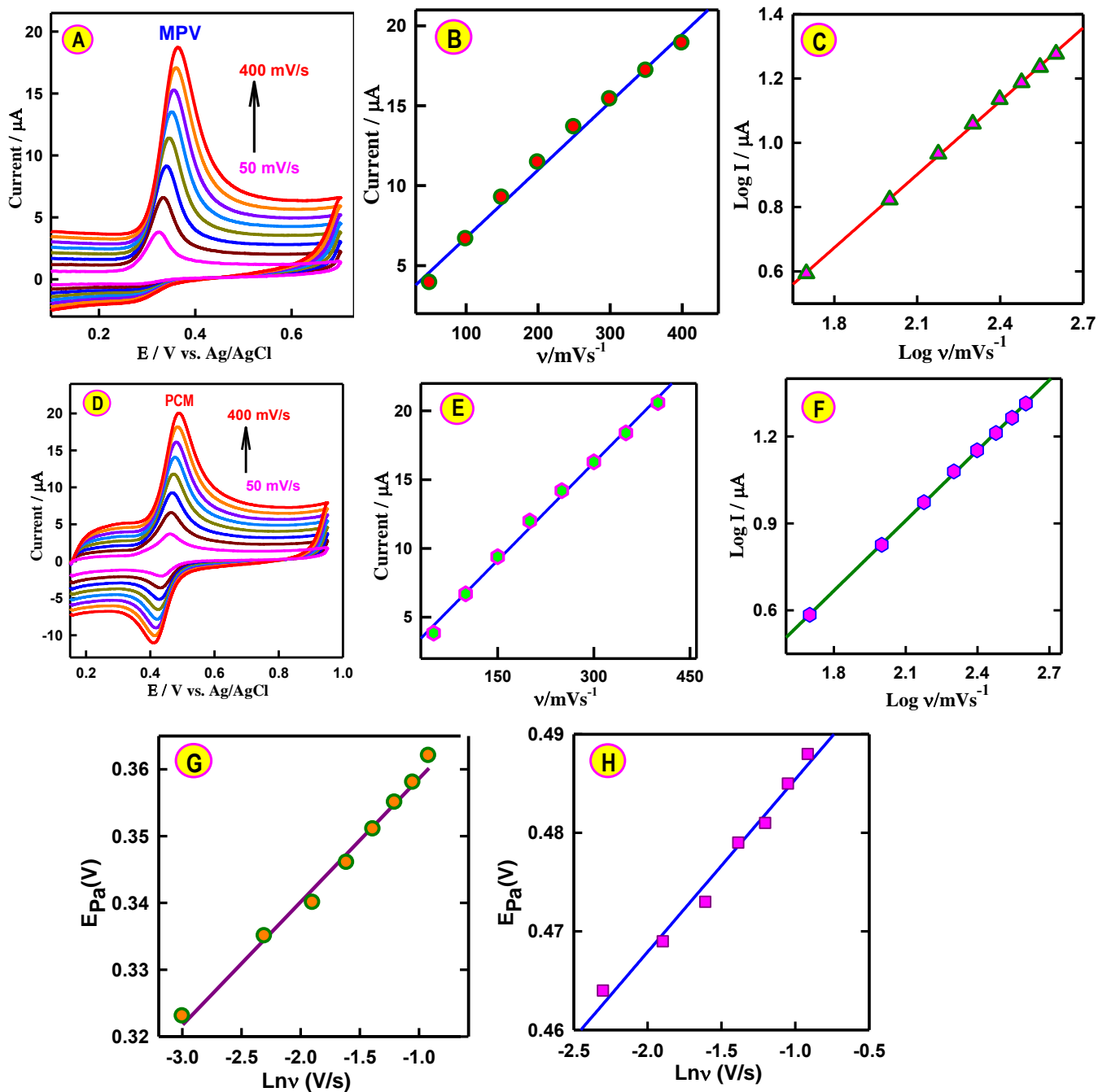
Fig. S6 (A) CVs of 1.5 µM MPV on surface of CeNPs@MWCNTs/GCMFE at various scan rates (50–400 mVs^{-1}) in BRB solution of pH 6.0. (B) Plot of $I_{Pa(MPV)}$ vs. ν. (C) Plot of Log $I_{Pa(MPV)}$ vs. Log ν. (D) CVs of 1.2 µM PCM on surface of CeNPs@MWCNTs/GCMFE at various scan rates (50–400 mVs^{-1}) in BRB solution of pH 6.0. (E) Plot of $I_{Pa(PCM)}$ vs. ν. (F) Plot of Log $I_{Pa(PCM)}$ vs. Log ν. (G) Plot of $E_{Pa\,(MPV)}$ vs. Ln ν (H) Plot of $E_{Pa\,(PCM)}$ vs. Ln ν.
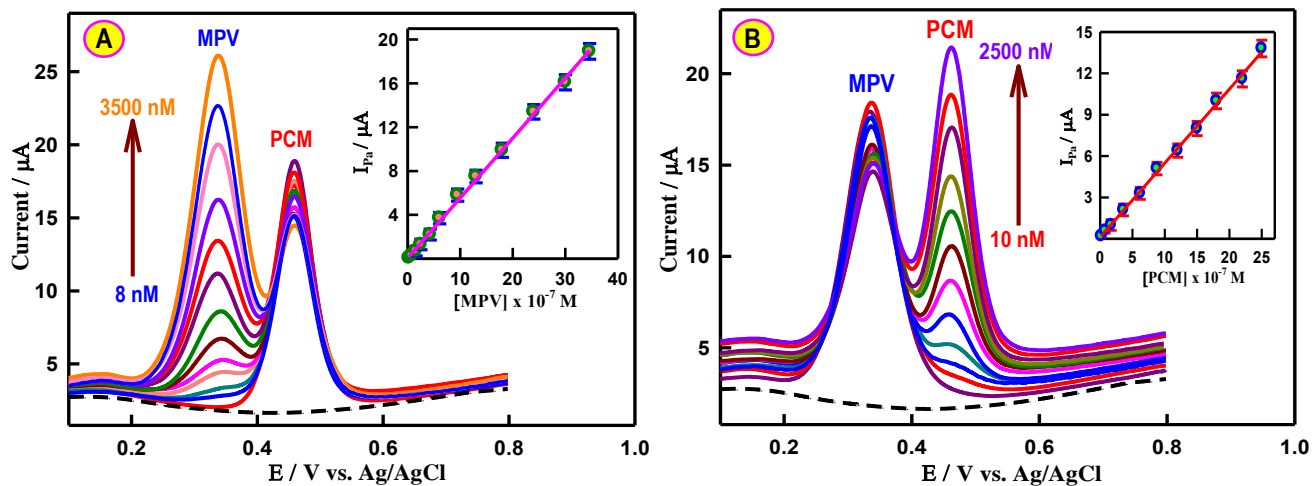
Fig. S7 (A) AdS-SW voltammograms of CeNPs@MWCNTs/GCMFE in BRB solution of pH 6.0 (B) with varied concentrations of MPV (8.0–3500 nM) in presence of 2.8 μM PCM and (B) with varied concentrations of PCM (10.0–2500 nM) in presence of 2.6 μM MPV.
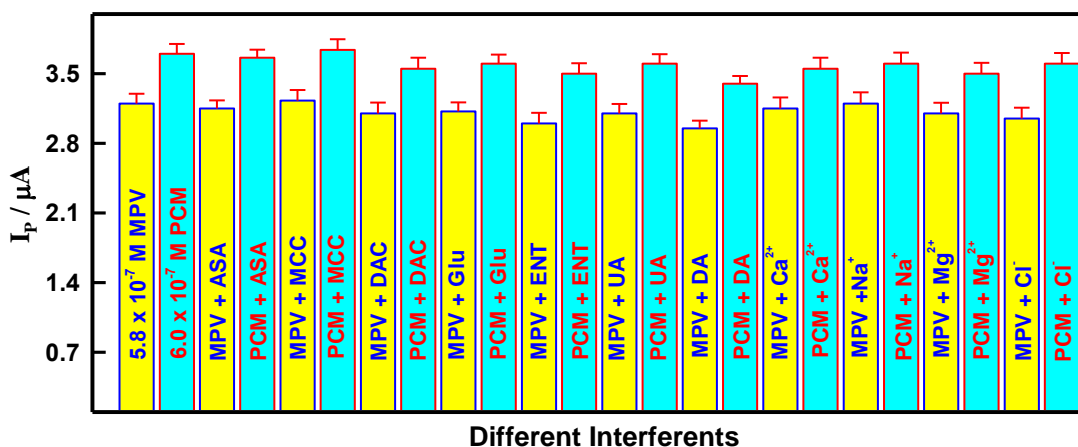
Fig. S8: Anti-interference ability of the CeNPs@MWCNTs/GCMFE sensor towards 5.8×10^{-7} M MPV and 6.0×10^{-7} M PCM monitoring in the presence of acetylsalicylic acid (ASA), microcrystalline cellulose (MCC), daclatasvir (DAC), glucose (Glu), entecavir (ENT), uric acid (UA), dopamine (DA), Ca^{2+}, Na^+, Mg^{2+}, and Cl^-.

Selectivity, reproducibility, and stability of CeNPs@MWCNTs/GCMFE

The anti-interference study was conducted in BRB solution (pH =6.0) with the addition of MPV and PCM and other compounds to be tested taken in 50-fold higher concentrations than MPV and PCM (Fig. S8). The compounds taken for selectivity analysis were acetylsalicylic acid (ASA), microcrystalline cellulose (MCC), daclatasvir (DAC), glucose (Glu), entecavir (ENT), uric acid (UA), dopamine (DA), Ca^{2+}, Na^+, Mg^{2+}, and Cl^-. From the obtained plot, only a slight difference in the responses of MPV and PCM can be observed. The excellent selectivity of the electrocatalyst modified GCMFE is evident with the results observed. Studies like the repeatability and reproducible behavior of the prepared sensor and its stability remain the foremost sensing features to be studied.
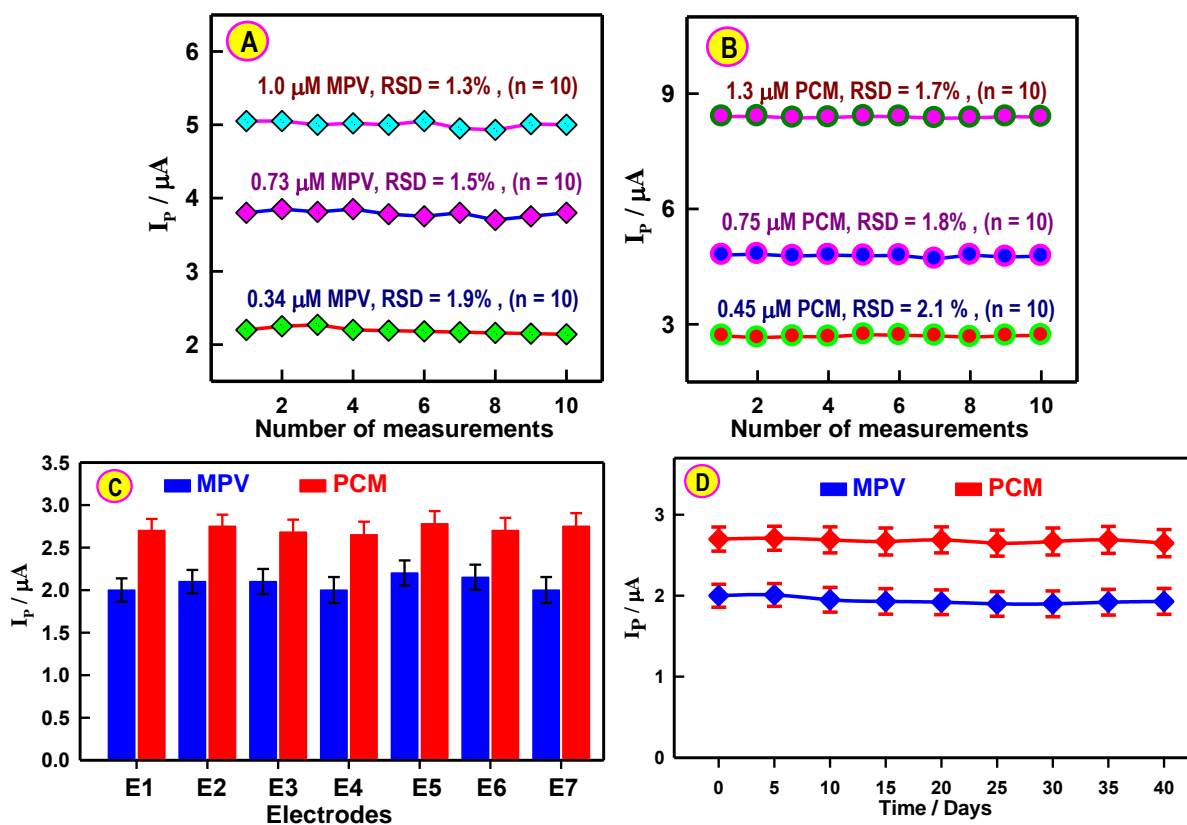
Fig. S9: The repeatability of MPV and PCM simultaneous determination with the CeNPs@MWCNTs/GCMFE sensor was evaluated from the peak current responses of ten measurements of MPV at concentrations of 0.34, 0.73 and 1.0 μM (A) and PCM at concentrations of 0.54, 0.75 and 1.3 μM (B). (C) Reproducibility studies of seven different CeNPs@MWCNTs/GCMFE sensors for 0.34 μM MPV and 0.54 μM PCM. (D) Long-term stability of CeNPs@MWCNTs/GCMFE sensor over 40 days.

The repeatability of the simultaneous detection of MPV and PCM using the CeNPs@MWCNTs/GCMFE was measured by monitoring the current response of three MPV and PCM concentrations ten times. The relative standard deviations (RSDs) of the current responses obtained from 0.34, 0.73 and 1.0 μM MPV, respectively, were 1.9%, 1.5% and 1.3% (Fig. S9 A). Similarly, The relative standard deviations (RSDs) of the current responses obtained from 0.45, 75 and 1.3 μM PCM, respectively, were 2.1%, 1.8% and 1.7% (Fig. S9 B). The reproducibility of the construction of the CeNPs@MWCNTs electrocatalyst modified GCMFE was evaluated by preparing six sensors at different times (Fig. S9 C). The RSD for the seven electrodes was determined to be 1.9% and 1.6% for MPV and PCM, respectively. Moreover, the long-term storage stability was analyzed by AdS-SWV in 0.34 μM MPV and 0.54 μM PCM using the fabricated sensor for 40 days at room temperature (Fig. S9 D). The

observed peak current reduced less than 5% of the initial signal after 40 days. These results imply that the fabricated CeNPs@MWCNTs/GCMFE has excellent repeatable measurement, reproducible preparation, and satisfactory stability for the simultaneous electrochemical detection of MPV and PCM.
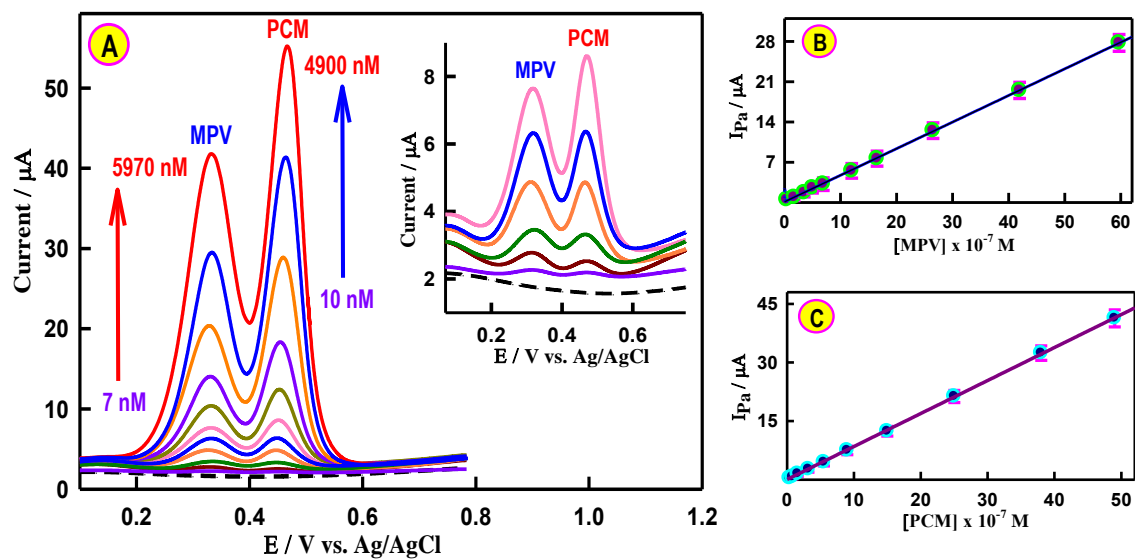
Fig. S10 (A) AdS-SW voltammograms of simultaneous determination of MPV (7.0 – 5970 nM) and PCM (10 – 4900 nM) spiked human serum sample at CeNPs@MWCNTs/GCMFE in BRB solution (pH 6.0). (B, C) Corresponding calibration plots for MPV and PCM. Error bar represents the standard deviation of triple measurements.

Fig. S11 (A) AdS-SW voltammograms of simultaneous determination of MPV (8.0 – 6000 nM) and PCM 9 – 4970 nM) spiked human urine sample at CeNPs@MWCNTs/GCMFE in BRB solution (pH 6.0). (B, C) Corresponding calibration plots for MPV and PCM. Error bar represents the standard deviation of triple measurements.
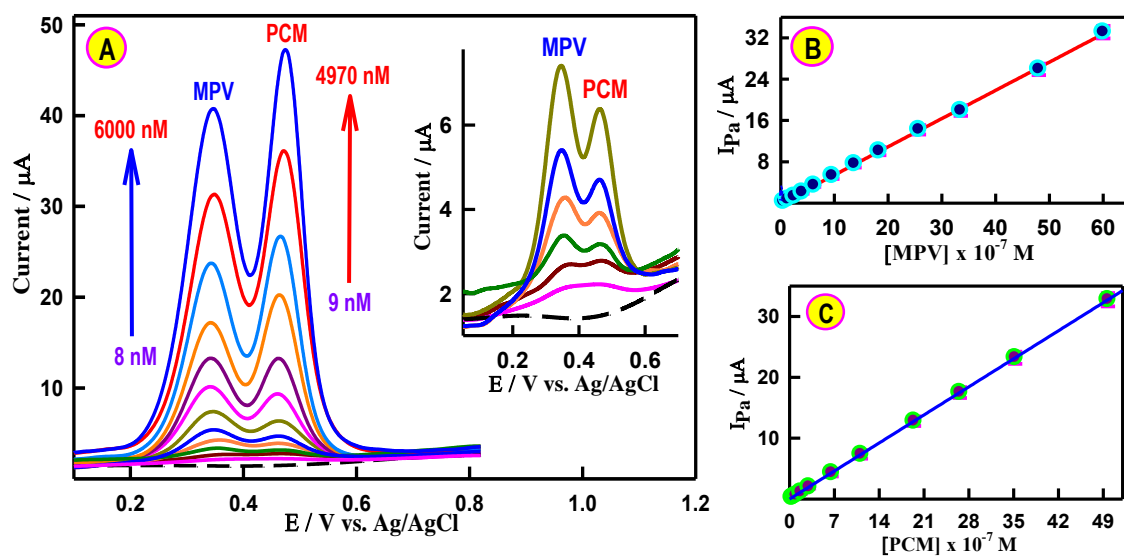
Table S1:

Electrochemical data of 5 mM $[Fe(CN)_6]^{-3/-4}$ in 0.1M KCl at different working electrodes

Electrodes	From EIS			From CV			
	R_{ct} $(\Omega)^a$	$k_{et} \times 10^{-4}$ (cm s^{-1})	I_o (μA cm^{-1})	ΔE_P (mV)	I_{Pa} (μA)	A_{eff} (cm^2)^a	R_f
GCMPE	3025 ± 53	2.50	8.5	454	59	0.19 ± 0.006	2.7
GCMFE	2225 ± 46	3.40	11.5	299	93	0.25 ± 0.010	3.6
CeNPs/GCMFE	1438 ± 38	6.0	17.8	223	113	0.33 ± 0.014	4.7
MWCNTs/GCMFE	963 ± 24	8.0	26.7	198	141	0.43 ± 0.017	6.1
CeNPs@MWCNTs/GCMFE	225 ± 13	33.8	114.0	161	218	0.86 ± 0.022	12.3

[a] Mean ± Standard deviation for n = 3.

R_{ct}, charge transfer resistance; k_{et}, the heterogeneous electron transfer rate constant; I_o, standard exchange current density; A_{eff}, effective surface area (cm^2); R_f, roughness factor.

Table S2:

Regression data of the calibration lines for simultaneous determination of MPV and PCM in standard solution at CeNPs@MWCNTs/GCMFE using AdS-SWV.

Parameters	MPV	PCM
Linearity range (nM)	5 – 5120	8 – 4162
Slope ($\mu A \mu M^{-1}$)	5.5	6.6
Standard error of slope	0.014	0.02
Intercept (μA)	0.06	0.01
Standard error of intercept	0.027	0.03
Coefficient of determination (R^2)	0.9985	0.9988
Standard error of estimate	0.12	0.08
Number of measurements (n)	3	3
LOD (nM)	6.0	8.6
LOQ (nM)	20	29
Sensitivity ($\mu A\ \mu M^{-1}\ cm^{-2}$)	78.6	94.3

LDR; linear dynamic range, LOD; limit of detection, LOQ; limit of quantitation

Table S3:

Regression data of the calibration lines for simultaneous determination of MPV and PCM in human serum and urine samples at CeNPs@MWCNTs/GCMFE using AdS-SWV.

Parameters	Serum		Urine	
	MPV	PCM	MPV	PCM
Linearity range (nM)	7 – 5970	10 – 4900	8 – 6000	9 – 4970
Slope ($\mu A \mu M^{-1}$)	4.6	8.4	5.3	6.6
Standard error of slope	0.017	0.03	0.021	0.02
Intercept (μA)	0.12	0.05	0.05	0.04
Standard error of intercept	0.05	0.04	0.08	0.02
Coefficient of determination (R^2)	0.9983	0.9979	0.9987	0.9985
Standard error of estimate	0.08	0.07	0.1	0.07
Number of measurements (n)	3	3	3	3
LOD (nM)	7.2	10.7	8.5	9.5
LOQ (nM)	24	36	28	32
Sensitivity ($\mu A \, \mu M^{-1} \, cm^{-2}$)	65.71	120	75.7	94.3

Table S4:

Analysis of MPV and PCM in its commercial tablets by AdS-SWV using CeNPs@MWCNTs/GCMFE

Sample	Spiked (μM)	Found[a] (μM)	RSD (%)	Recovery (%)
Molnupiravir-Rameda 200 mg	0.0	0.8	2.5	-
	0.75	1.5	1.9	96.8
	1.4	2.25	1.6	102.3
	2.8	3.5	1.8	97.2
Panadol 500 mg	0.0	1.65	2.2	-
	1.6	3.20	2.9	98.5
	2.5	4.05	1.8	97.6
	3.6	5.34	2.4	101.7

[a]Average of five determinations at optimum conditions