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Full Paper

Simultaneous Determination of Acetaminophen in the Presence of Adrenaline at BiVO₄/MCPE: A Cyclic Voltammetry Study

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Abstract- In the present work, the BiVO₄ nanoparticles (BiVO₄/NPs) were synthesized using the co-precipitation method and characterized by the X-ray diffraction (XRD), scanning electron microscope (SEM) and energy dispersive spectroscopy (EDS) techniques. The BiVO₄/NPs were utilized as electrode modifiers with carbon paste electrode (BiVO₄/MCPE), as-prepared modified electrode surface was pretreated with and the NaOH (BiVO₄/NaOH/MCPE). The pretreated modified electrode was used for the simultaneous determination of acetaminophen (ACOP) and adrenaline (AD) by using cyclic voltammetry (CV) and differential pulse voltammetric (DPV) techniques. The influence of variation of concentration of ACOP and AD was investigated at BiVO₄/NaOH/MCPE. The limit of detection (LOD) and limit of quantification (LOQ) of both ACOP and AD at BiVO₄/NaOH/MCPE, respectively were determined, the LOD was found to be 3.3 µM for ACOP and 5.9 µM for AD, and LOQ was found to be 11.1 µM for ACOP and 17.8 µM for AD, respectively. Moreover, the BiVO₄/NaOH/MCPE is used for the sensitive and selective determination of ACOP and AD in real samples.

Keywords- BiVO₄; Nanoparticles; Modified carbon paste electrode; Cyclic voltammetry; Paracetamol

1. INTRODUCTION

In the research field, nanotechnology is rapidly growing and nanoparticles were much interested in the industry and electrochemical applications due to their many interesting properties including catalytic, electronic, mechanical, optical, and biological [1,3]. In addition, nanoparticles were used in anticancer agents, antidiabetics, imaging agents, and biosensor studies [4-7]. Among the numerous nanoparticles, bismuth vanadium oxide (BiVO₄) nanoparticles are of great interest owing to their properties as well as their wide applications, it is an n-type semiconductor, and its great importance in ferroelastic, pyro-optical, ionicconductivity, catalytic, inorganic dyes, absorbent and sensor applications [8]. Moreover, the electrochemical activity of BiVO4 directly depends on crystalline structures such as monoclinic and tetragonal. However, a monoclinic structure of BiVO₄ shows excellent electrocatalytic activity [9], BiVO₄ is used in commercially available organic pigments [10]. Over the last few decades, the vanadium metal oxides have been exposed as a good catalyst [11-15], a monoclinic structure of BiVO₄ synthesized using different methods such as precipitation, hydrothermal [16], sol-gel [17], and chemical bath deposition [18]. In our work, the BiVO₄ nanoparticle was prepared by the co-precipitation method. BiVO₄ nanoparticle was widely used in biosensor applications, due to their broad range of redox reaction at the surface of electrodes [19].

Acetaminophen (ACOP) / Paracetamol / N-acetyl-p-aminophenol is commonly used as a pain killer and fever reducer, it is classified into two types such as antipyretics and analgesics drugs, ACOP is a white crystalline powder with melting point of 169° C, it is weak acid having pK_a value of 9.5 [20,21], ACOP widely administrated with postsurgical pain and post-operative pains [22]. advantageous over non-steroidal anti-inflammatory drugs (NSAIDs) [23]. The development of a sensitive and selective method for the simultaneous determination of ACOP side effects is desirable [24,25].

Adrenaline (AD) is also known as epinephrine [26], one of the most important hormones and medications in visceral functions, AD is used for stimulating the heartbeat and contracting blood vessels. Therefore, AD is an important role in medicine [27-29], it can determine by using several techniques such as HPLC [30], liquid chromatography [31], fluorimetry [32], flow injection [33], chemiluminescence [34]. However, these techniques suffer from several disadvantages and high cost and time analysis, nowadays electrochemical sensor methods were more attractive due to their sensitivity, selectivity, lowest cost, and accuracy.

Herein, we reported the synthesis of BiVO₄ nanoparticles and the method of pre-treatment using NaOH on the surface of BiVO₄/MCPE. The prepared BiVO₄/NaOH/MCPE has demonstrated superior electrocatalytic activities for the oxidation of ACOP and AD, individually and simultaneously. Further, the BiVO₄/NaOH/MCPE was used for the stability, sensitivity, and selectivity studies. The fabricated electrode has efficient to perform good recovery in real samples of commercially available ACOP.

2. EXPERIMENTAL SECTION

2.1. Reagents

Bismuth nitrite penta hydrate $[Bi(NO_3)_3.5H_2O]$, ammonium vanadate (NH_4VO_3) , chemicals were received from Merck, nitric acid (HNO_3) , sodium hydraoxide (NaOH), acetaminophen (ACOP) and adrenaline (AD) were obtained from Sigma-Aldrich, potassium chloride (KCl) and potassium ferrocyanide K₄[Fe(CN)₆] were brought from SD-Fine chemicals, and its solutions were prepared in distilled water. AD stock solutions were prepared in 0.1 M perchloric acid. Graphite powder from Merck chemicals and silicon oil was purchased from Himedia, 0.2 M phosphate buffer solutions were prepared from monosodium dihydrogen phosphate (NaH₂PO₄) and disodium hydrogen phosphate (Na₂HPO₄). Double distilled water was used for the preparation of all the solutions.

2.2. Apparatus

The CV and DPV manner were performed on model CHI-660c. The bare carbon paste electrode (BCPE) and BiVO₄/MCPE and BiVO₄/NaOH/MCPE were acting as working electrodes, the saturated calomel electrode (SCE) platinum wire is used as reference and counter electrodes. XRD measurement was performed in model number XPERT-PRO, the morphology of synthesized material was observed by using SEM model number IRA3 TESCAN, and EDS analysis was studied on an APEX-009 spectrometer.

2.3. Preparation of BiVO₄ NPs

The preparation of BiVO₄ nanoparticle was done by the co-precipitation method, at first Bi(NO₃)₃.5H₂O was blended in concentrated HNO₃. NH₄VO₃ was prepared separately with 50 ml of distilled water, Then two solution was mixed using the hot magnetic stirrer for 1 hour at 60 °C temperature after that 0.1 M NaOH solution was added dropwise to the beaker containing the mixture solution i.e Bi(NO)₃ and NH₄VO₃ placed in a hot magnetic stirrer at 80 °C for 4 hours. Finally, the yellow precipitate was obtained, and ethanol was added to remove the impurities. The wet precipitate was collected and dried with calcinated at 600 °C in a furnace to obtain the pure monoclinic BiVO₄ [35].

2.4. Fabrication of electrodes

The BCPE was prepared by mixing graphite powder and silicone oil thoroughly in an agate mortar for 30 minutes to form a homogeneous paste. The MCPE was prepared by the same method. However, 6mg of BiVO₄/NPs were added to the carbon paste. The BiVO₄/NaOH/MCPE was prepared by pretreatment of 0.1 M NaOH on the BiVO₄/MCPE surface by CV for 15 cycles at applied potentials from 0.1 to -0.6 and scan rate 50 V s⁻¹ [36] as shown in Figure 1A. It shows the CVs obtained for the pretreatment process, After, the

pretreatment, the electrode was washed with double distilled (DD) water. The plot of anodic peak current versus the number of pretreated NaOH cycles were shown in Figure 1B. From the graph, anodic peak currents increased gradually from five to fifteenth cycles, this expresses the formation and development of an electroactive layer of NaOH on the surface of BiVO₄/MCPE. Later the fifteenth cycle decrease in peak current response at the twentieth cycle, and film thickness affects the electrocatalytic properties of the carbon paste electrode. However, a better catalytic performance was observed in the fifteenth-cycle scan. Therefore, the fifteenth-cycle scan was chosen for the study of all other parameters.

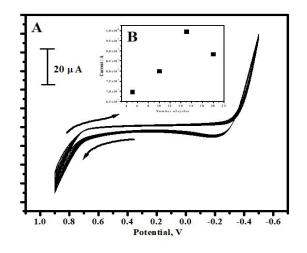


Figure 1. A) Electrochemical pretreatment of BiVO₄ nanoparticle in 0.2 M NaOH with scan rate 50 mV/s electrochemical pretreatment at 15 multiple cycles; B) the plot of anodic peak current versus a number of cycles

3. RESULTS AND DISCUSSION

3.1. Characterization of prepared nanoparticles

The XRD pattern of the synthesized BiVO₄ nanoparticle is shown in Figure S1 (supplementary file), the XRD peaks showed the high crystalline nature of the synthesized sample, all peaks can be accurately correlated to the monoclinic structure, and there is good agreement with the reference (JCPDS card no. 75-1866). Scherer's equation was used to determine the average crystalline size [37]:

$$d = \frac{k\lambda}{\beta\cos\theta} \tag{1}$$

where K is the Scherrer's constant for the hexagonal system, θ is the Bragg diffraction angle, β is the FWHM of the diffraction peak, and λ is the wavelength of the incident X-rays (0.15406 nm). It was observed that the average size was 26 nm. The average size was found to be 26 nm. The morphological structure of the prepared BiVO₄ nanoparticle was confirmed by SEM. In Figure S2A (supplementary file) the images showed the rice-shaped morphological

arrangement of the prepared BiVO₄ nanoparticle with an approximate particle size of 20 nm. In Figure S2B, the EDS spectrum of BiVO₄ nanoparticles confirms the elements of bismuth (Bi), vanadium (V), and oxygen (O). However, carbon appeared may be because of calcination at the end of the synthesis process.

3.2. Electrochemical characterization of Bare and modified CPE electrodes

The bare and modified CPE were utilized to study the electrochemical properties of $K_4[Fe(CN)_6]$. The CV technique was used to summarize both electrode results. Electrochemical response for the bare-CPE (curve-a), BiVO₄/MCPE (curve-b), and BiVO₄/NaOH/MCPE (curve-c) were characterized in the presence of 10µM K₄[Fe(CN)₆] at the sweep rate 50 mV/s in 0.1 M KCl solution, respectively. Figure 2 depicts less sensitivity of the BCPE (curve a) and obtained peak potential is Ep_a=0.256 V, comparatively BiVO₄/MCPE (curve b) has shown more enhancement in sensitivity with the peak potential Ep_a=0.243 V, because, BiVO₄ nanoparticle increased the more surface area on the CPE.

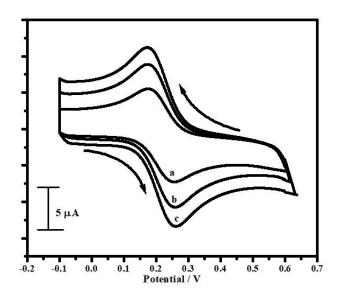


Figure 2. CVs of 10µM K4[Fe(CN)6] in 0.1 M KCl at different modified electrodes

As seen from curve-c, the BiVO₄/NaOH/MCPE is accompanied by a more increase in the peak current feedback, and obtained peak potential was 0.234 V. Therefore, the enhancement factor in anodic peak current and shifting of anodic peak potential at the BiVO₄/NaOH/MCPE exhibited the excellent sensitivity compared to the other mentioned two electrodes due to the electrocatalytic effect of NaOH molecule on the surface of BiVO₄/MCPE, which enhanced more electroactive site. The electro-active surface area of the electrodes was studied in the presence of K₄[Fe(CN)₆] at 0.1 M KCl by the Randles-Sevcik equation:

$$I_p = 2.69 \times 10^5 \text{ n}^{3/2} \text{ AD}^{1/2} \text{ C}^{\circ} \text{v}^{1/2}$$
(2)

where, I_p is the peak current in A, D is the diffusion coefficient (cm²s⁻¹), C is the concentration of the electroactive species (mol cm⁻³), n is the number of electrons exchanged, v is the scan rate (V s⁻¹) and A is the electroactive surface area (cm²) [38,39]. The active surface area of the BCPE, BiVO₄/MCPE, and BiVO₄/NaOH/MCPEs were found to be 0.234, 0.282, and 0.333 cm², respectively. The surface area increased following orders BiVO₄/NaOH/MCPE> BiVO₄/MCPE>BCPE, it can be concluded that BiVO₄/NaOH/MCPE is more efficient for electron transfer than BiVO₄/MCPE and BCPE.

3.3. pH variation study for ACOP at BiVO₄/NaOH/MCPE

The electrochemical oxidation of ACOP is significantly influenced by the pH 6.2 to 7.8 of the supporting electrolyte. Here, evaluated the pH effect on the oxidation of 10 μ M ACOP at BiVO₄/NaOH/MCPE using the CV technique. Figure 3A indicates the change in the peak current during the oxidation of ACOP when the pH of the solution increased from 6.2 to 7.8, and also shifts in peak potential toward negative value were observed, the peak current response ACOP increased with increasing the pH up to 7.4 and then decreased with further increase of pH value.

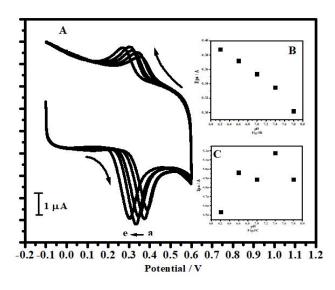
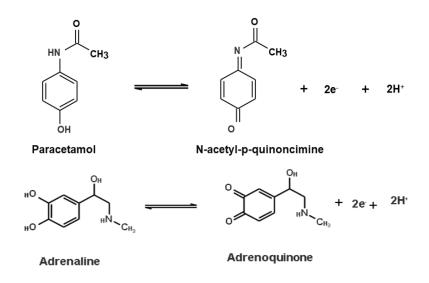


Figure 3. A) Variation of Ep_a in the presence of 10µM of ACOP in different pH solutions; B) Graph of Ep_a *vs.* pH; C) Graph of Ip_a *vs.* pH

The good linear relationship between the peak potential and pH of the solution was plotted in Figure 3B. The linear regression equation is R^2 =0.9882. The plot of anodic peak potential versus peak current was shown in Figure 3C. The result shows the anodic peak current of the first two peaks slightly increases from 6.2 to 6.8, then shortly decreases peak current at 7.0. After that sharply increases peak current reaches a maximum at pH 7.4 and decreases peak current by increasing the pH value at 7.8. which indicates the participation of an equal number of protons and electrons involved in the reaction mechanism as shown in Scheme 1.



Scheme 1. Oxidation of paracetamol and adrenaline

3.4. Electrochemical investigation of ACOP and AD at BiVO₄/NaOH/MCPE

Figure S3 depicts the CVs for the 10 μ M ACOP in the presence of buffer solution (pH 7.4) as a supporting electrolyte at BCPE and BiVO₄/NaOH/MCPE with a scan rate of 50 V s⁻¹. As observed from the results confirmed that both anodic and cathodic peak potential signals at BPCE (solid line) are 0.342 V. The corresponding peak potential signals at BiVO₄/NaOH/MCPE are 0.283 V. The overall result shows the presence of BiVO₄/NaOH/MCPE (dotted line) decreases the overpotential and increases the peak current response. Similarly, for AD, Figure S4 at the BCPE (dotted line) shows the poor current response and obtained peak potential at 0.234 V, and BiVO₄/NaOH/MCPE (solid line) shows the excellent increment of peak current response and decreases in the peak potential response are 0.198 V. Therefore, the BiVO₄/NaOH/MCPE shows a better sensitivity as compared to the BCPE.

3.5. Sweeping rate effect of ACOP and AD at BiVO4/NaOH/MCPE

A concentration of 10 μ M ACOP was used for the investigation of the sweeping rate effects (Figure 4A). As can be seen, the anodic peak current (Ip_a) response increases with increasing the sweep rate ranges from 50-500 Vs^{-1,} and the anodic peak potential shifts to the less negative side. Figure 4B, the graph of anodic peak current versus sweep rate was plotted, and the MCPE exhibited a good linearity and good correlation coefficient value (R²=0.9963) and Figure 4C showed a plot of the square root of sweep rate versus Ip_a with R²=0.9837. The obtained results suggested that the adsorption-controlled electrode process of BiVO₄/NaOH/MCPE, AD

behavior on BiVO₄/NaOH/MCPE was tested by CV (Figure 5A), at various sweep rates in pH 7.4, the anodic sweep rate was proved that the electrode process is irreversible. In Figure 5B, a linear relation was obtained between peak current versus sweep rate with the correlation coefficient value R^2 =0.9916 and the peak current versus square root sweep rate was plotted as shown in Figure 5C with correlation coefficient value R^2 =0.9974, respectively, which is attributed to adsorption controlled process.

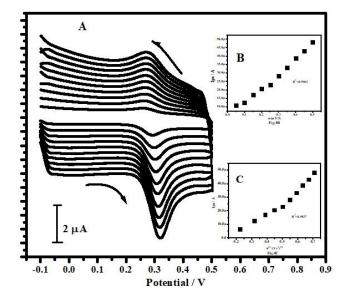


Figure 4. A) CVs of 10 μ M ACOP at BiVO₄/NaOH/MCPE in 0.2 M PBS at different sweep rates from 50 to 500 Vs⁻¹; B) Linear relationship between Ip_a *vs*. sweep rate; C) Variation of Ip_a *vs*. square root of sweep rate

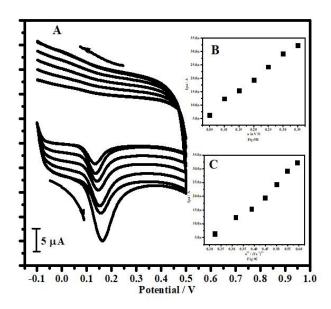


Figure 5. A) CVs of 10 μ M AD at BiVO₄/NaOH/MCPE in 0.2 M PBS at different sweep rates from 50 to 350 Vs⁻¹; B) Linear relationship between Ip_a *vs*. sweep rate; C) Variation of Ip_a *vs*. square-root of sweep rate

3.6. Concentration study for ACOP and AD at BiVO4/NaOH/MCPE

Figure 6A depicts the cyclic voltammograms of various concentrations of ACOP carried out in 0.1 M PBS (pH 7.0) at BiVO₄/NaOH/MCPE, the peak current increment linearly against the concentration of ACOP with the range of 10-100 μ M, the graph of Ip_a versus concentration of ACOP was plotted in Figure 6B, it shows the good linearity with obtained correlation coefficient value R²=0.996. The LOD and LOQ were calculated by using equation LOD= 3S/M [40] and LOQ=10S/M [41], (Tables 1 & 2) where "S" stands for standard deviation and "M" for slope, both of which were determined from the calibration plots to be 3.3 M and 11.1 M, respectively.

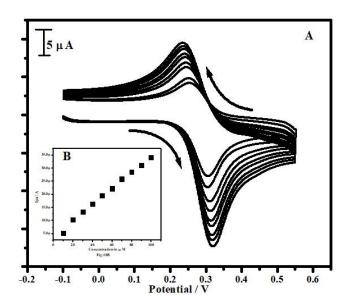


Figure 6. A) CVs of 10μ M ACOP at BiVO₄/NaOH/MCPE in 0.2 M PBS with various concentrations (10-100 μ M); B) graph of Ip_a *vs.* varied concentration of ACOP

Table 1. Comparison LOD values of BiVO₄/NaOH//MCPE with other electrodes for detection of PA

Sl. No	Electrode	Detection limit (µM)	Technique	References
		(µ101)		
1	PVA/ Fe ₃ O ₄ /MGCPE	8.0	DPV	[42]
2	MgO/TX-100/MCPE	6.2	CV	[43]
3	Gr/RAC sensor	8.3	DPV	[44]
4	TiO ₂ /MCPE	5.2	CV	[45]
5	BiVO ₄ /NaOH/MCPE	3.3	CV	This work

Figure 7A, in order to estimate the effect of AD concentration on BiVO₄/NaOH/MCPE in the presence of pH 7.4, concentrations varied in the range 10 to 100 μ M. The result is shown in Figure 7B, the graph of anodic peak current versus concentration of AD shows good linearity and obtained with a good correlation coefficient value R²=0.9998, the detection limit was found to be 5.9 μ M and the limit of quantification was found to be 17.8 μ M, respectively. Table 1 and Table 2 summarize the analytical performance of the different modified electrodes for the determination of ACOP and AD, obtained results showed acceptable sensitivity and good current response for ACOP and AD, respectively.

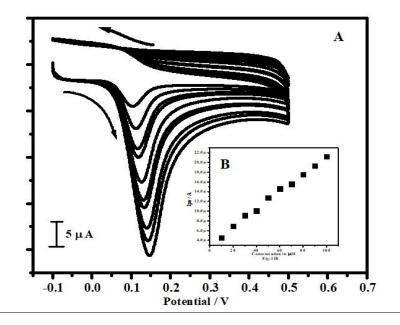


Figure 7. A) CVs of 10 μ M AD at BiVO₄/NaOH/MCPE in 0.2M PBS with various concentrations (10-100 μ M); B) graph of Ip_a *vs.* varied concentration of ACOP

 Table 2. Comparison LOD values of BiVO4/NaOH//MCPE with other electrodes for detection of AD

Sl. No	Electrode	Detection limit (µM)	Technique	References
1	GCE/MWCNT/poly-FA	22	CV	[46]
2	Gold film electrode	19	CV	[47]
3	Poly (allura red) MCPE	6.8	CV	[48]
4	Au 4MpyAuNPs	4.5	CV	[49]
5	BiVO ₄ /NaOH/MCPE	5.9	CV	This work

3.7. Simultaneous study

Simultaneous determination of ACOP and AD at BiVO₄/NaOH/MCPE and BCPE were executed by CV. Figure S5, illustrates the equimolar mixture of ACOP and AD, at the BCE shows a low current signal, less sensitivity, and selectivity with an obtained peak potential of 0.342 V for ACOP and 0.145 V for AD respectively. Similarly, at the MCPE ascribed higher peak current with the peak potential of 0.310 V (ACOP) and 0.109 V (AD) respectively, hence suggesting that simultaneous determination of these compounds developed at BiVO₄/NaOH/MCPE are independent, these mixtures were determined without any significant interference.

3.8. Interference studies

DPV technique was selected for the ACOP in the presence of the AD solutions, Figure S6A, represents the DPV of ACOP in the presence of AD, the selectivity of BiVO₄/NaoH/MCPE of varying the ACOP concentration ranges from 10-100 Vs⁻¹ and keeping the AD concentration while kept constant, reveals that the increase in the concentration with increases the peak current of ACOP. Similarly, in Figure S6B, the concentration of AD varying from 10-100 Vs⁻¹ and keeping the ACOP concentration, these results exhibited the BiVO₄/NaOH/MCPE shows good linearity. Hence, it can be concluded that the MCPE has shown excellent sensitivity and selectivity toward the determination of ACOP and AD.

3.9. Reproducibility and stability

The electrode has demonstrated good consistency through 15 cycles of testing the standard method for evaluating ACOP in the presence of 0.2 M of 7.4 pH containing 10 μ M ACOP. Following each determination, the modified electrode surface was cleaned with PBS and scanned with CV in the empty PBS until the redox response wave of the ACOP vanished at 50 m Vs⁻¹. The electrochemical activity of the BiVO₄/NaOH/MCPE over the determination of ACOP was found to be nearly unchanged after one week of exposure to air, indicating strong reproducibility and stability of the BiVO₄/NaOH/MCPE.

3.10. Real sample analysis

To evaluate the practical applicability of BiVO₄/NaOH/MCPE, the ACOP tablet was used as the real sample for the analysis. The ACOP tablets received from Mark Laboratories Ltd are diluted with 0.2 M PBS (pH 7.4). The analysis results are shown in Table 3. The collected recovery range is 95 to 98%. These results indicate that the purposed sensor can be used to determine the ACOP in real sample analysis.

Sample	PA added (µM)	Found (µM)	Recovery (%)
PA tablet	10	9.85	95.93
	20	18.62	95.52
	30	28.13	98.12

Table 3. Detection of PA in tablet sample at BiVO₄/NaOH//MCPE (n=3)

4. CONCLUSION

The BiVO₄ nanoparticle was symphonized by the co-precipitation method and was characterized by XRD, SEM, and EDS manners, the minded BiVO₄ nanoparticle was used as MCPE with pretreatment of NaOH. The prepared BiVO₄/NaOH/MCPE shows a significant enhancement of electrocatalytic activity towards ACOP and AD, the proposed electrode showed good linearity and kinetic electron transfer reaction, and the concentration study shows that the wide range of LOD was found to be $3.3 \,\mu$ M for ACOP and $5.9 \,\mu$ M for AD, respectively. Similarly, LOQ was found to be $11.1 \,\mu$ M for ACOP and $17.8 \,\mu$ M for AD, The BiVO₄/NaOH/MCPE can be used to build a sensitive and selective measurement of various significant biological molecules.

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