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Development of a sensitive and inexpensive electrochemical sensor for indigotin using poly(valine) modified carbon paste electrode

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ABSTRACT

In this study, an electro-polymerized valine (VLN) stimulated carbon paste electrode (CPE) was used to create a straightforward, inexpensive, and renewable electrochemical sensor for accurate and selective indigotin (IGN) determination. Comparing the CPE, to the modified electrode, it exhibits excellent sensibility for the IGN oxidation-reduction reaction. Multiple techniques, including cyclic voltammetry (CV), differential pulse voltammetry (DPV), electrochemical impedance spectroscopy (EIS), and scanning electron microscopy (SEM) were utilized in this case to characterize the electrode materials. IGN was analyzed using CPE and poly(valine) modified carbon paste electrodes (P(VLN)MCPE) taking a 6.5 pH in 0.2 M phosphate buffer solution (PBS). Because it has more active spots than the CPE and a strong electrocatalytic nature, P(VLN)MCPE exhibits excellent electrochemical performance. The impact of pH, scan rate, numerous interferents, and fluctuation in analyte concentration were only a few of the important electrochemical factors that were investigated. The variation in scan rate proves that the IGN oxidation-reduction reaction on the surface of P(VLN)MCPE is as follows an adsorption-controlled pathway. The P (VLN)MCPE displays a good electrochemical nature for IGN in the 0.2 to 5.0 µM range, with a low limit of detection (LOD) is 0.0069 μ M and a limit of quantification (LOQ) is 0.023 μ M. P(VLN) MCPE shows good reproducibility, stability, and repeatability for the detection of IGN. Additionally, P(VLN)MCPE's analytical applicability for IGN detection in water sample was assessed with impressive recovery.

1. Introduction

IGN is a blue organic compound used as a colorant and a significant food additive, obtained from natural sources or synthesized in laboratories. Artificial colorants are used in many applications like medicine, food, cosmetics, and even in dyeing of polyester fibers. Generally, dyes are added to achieve specific coloration, enhancing the appeal of products such as food items, paper, leather, electronics, photographic films, textile, paints, plastic, etc [1–3]. In the laboratory, IGN serves as an analytical indicator in chemistry, and a

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biological stain in microscopic observations [4].

IGN is an extremely impure colorant, often containing substantial impurities present in wastewater. This contamination negatively affects aquatic plants and animals living in the water resources [5]. Natural colors are inherently unstable and tend to degrade during food processing and packaging. Consequently, artificial colors have been employed for many years in food production to replace unstable natural types [6]. Synthetic colors offer several advantages over natural ones, including better stability, under light, varying pH, and exposure to oxygen, consistent color intensity, good water solubility, minimal bacterial contamination, and cost-effective [7]. nonetheless, the degradation of artificial colorants can be toxic and hazardous to living organisms [8,9]. Current research suggests that IGN is potentially hazardous to human health, causing lasting damage to the cornea and conjunctiva [10]. Moreover, IGN may induce genetic injury to cells; continued usage has been liked to mutagenesis, tumor formation, and cancer development [11,12]. Thus, monitoring and timely identification of IGN content in food ingredients are crucial.

Numerous analytical methods are employed for IGN analysis, such as reversed-phase high-performance liquid chromatography [13], spectrophotometry [14], thin-layer chromatography [15], and liquid chromatography with mass spectrometry [16]. Compared to these methods, electroanalytical methods are used for the analysis of IGN, outstanding in their simplicity, portability, lower price, and quick analysis time [17,18]. Cyclic voltammetry (CV) is a novel method in electrochemical technique based on a potential variation, particularly effective in studying redox reactions [19]. It stands out as an effective electrochemical method for compound analysis [20–22].

CPE are commonly used in electrochemistry due to their excellent electrochemical properties, such as low-cost, large active surface area, good electrical conductivity, thermal conductivity, and mechanical durability [23]. In this study, a CPE is employed for the electrochemical analysis of IGN due to these properties. Electrochemical methods provide strong conductivity, sensitive, and constant voltammetric responses for bioactive composites on the conductive amino acid-based modified electrode surface. Electrochemical-polymerization amino acids on electrode surfaces are widely used as biological modifiers due to their sensitivity, repeatability, reproducibility, and stability. Consequently, a P(VLN)MCPE is fabricated for IGN detection.

The current study developed a simple, subtle, and stable electrochemically polymerized sensor P(VLN)MCPE for IGN analysis. The CV, DPV, and EIS techniques were employed to examine the electrochemical behavior of the developed electrode materials for IGN detection. The interfering effects on the developed electrode were assessed by examining IGN detection in the presence of various metal ions and organic compounds. As per the literature survey, the newly developed sensor P(VLN)MCPE is being used for the first time for IGN sensing applications.

2. Materials and methods

2.1. Instrumentation

The electrochemical sensor CHI-6038E instruments (from the USA). Was utilized for the analysis, using the CV, DPV, and EIS, methods within its workspace. The trio-electrode arrangement was connected to the electrochemical analyzer and a computer for data storage. In this arrangement, the BCPE and P(VLN)MCPE were used as working electrodes, though a saturated calomel electrode (SCE) served as the reference electrode, and platinum cable was used as the auxiliary electrode. All research work was conducted at the lab temperature of 25 °C. The SEM images were provided by the DST-PURSE Laboratory, Mysore University, Karnataka, India.

2.2. Chemicals and substances

All the chemical substances were utilized without extra purification. IGN and carbon powder were purchased from the Tokyo Chemical Industry (Japan). Silicone oil and VLN were purchased from Molychem (India). Monosodium hydrogen phosphate monohydrate (NaH₂PO₄.H₂O), Disodium hydrogen phosphate dihydrate (Na₂HPO₄.2H₂O), and potassium ferrocyanide trihydrate K₄[Fe (CN)₆] were purchased from Himedia (India). Potassium chloride (KCl) was obtained from Nice Chemicals (India). The necessary reagents were prepared with a known quantity of chemicals in a specified amount of purified water.

2.3. Preparation of electrodes

Carbon powder was taken in a ratio of (70 %) and silicone oil in a ratio of (30 %) and the two were mixed well for about 10–15 min until a homogeneous paste was formed. This homogeneous paste was carefully filled into the cavity of the Teflon tube with a 3 mm internal space. The electrode surface was polished using tissue paper to develop a uniform surface, this is called BCPE. The electric connection was established by connecting a copper cable to the paste through a Teflon tube. The external electrode was revived before each measurement. The P(VLN)MCPE was prepared based on electro-polymerization of P(VLN) onto the surface of CPE in 0.2 M PBS with a pH of 6.5. this was achieved by performing 10 CV cycles at the scan rate of 0.1 V/s.

3. Results and discussions

3.1. Optimization of electrochemical polymerization cycles

The electrochemical polymerization of VLN was done at the BCPE surface, and the number of electrochemical polymerization cycles was enhanced to become electrocatalytic determination for IGN. Therefore, we changed the number of CV cycles to 5, 10, 15, 20,

and 25, and here 10 CV cycles show the highest current responses for IGN (Fig. 1a). Advanced, 10 CV cycles were chosen as the finest for the electrochemical polymerization of BCPE for all experimentation. Electrochemical polymerization (10 cycles) of 1.0 mM VLN in 0.2 M PBS with pH 6.5 at the scan rate of 0.1 V/s on CPE was finished using the CV method and the plotted graph is shown in Fig. 1b. This shows the change of a monomer of VLN into a polymer at the surface of the CPE electrode. Afterward, the finish of the electrochemical polymerization method and the electrode was rinsed using distilled water and study more analysis steps.

3.2. SEM study

The SEM technique was used to study a morphological feature of the BCPE and P(VLN)MCPE, and the SEM pictures are revealed in Fig. 2a, b, and 2c. In Fig. 2a, only the erratically distributed sheets-like shapes are observed which are due to the presence of carbon in BCPE. While, Fig. 2b surface shows a cloud like surface structure on the surface of CPE with more porous spots, and it reprints the P (VLN)MCPE. Additionally, Fig. 2c shows the surface morphology of the modified electrode after the electrochemical reaction of IGN at its surface, here totally different surface structure is observed (randomly distributed irregular shapes).

3.3. EIS study

The created electrode material's conductance and charge transfer resistance (Rct) are studied in the EIS investigation. In this study, 1 mM K₄[Fe(CN)₆] in 0.1 M KCl was used as a supporting electrolyte for the characterization of BCPE and P(VLN)MCPE using EIS. Fig. 3 displays the plots of the Nyquist plots for BCPE (curve-a) and P(VLN)MCPE (curve-b). In comparison to the BCPE, the P(VLN) MCPE offers a smaller semicircle. The Rct is directly proportional to the semicircle size. As a result, the created electrode has a higher conductivity and a lower R_{ct} than the BCPE. Also, the equivalent circuit was fixed based on the Nyquist plot holding the following factors like R_{ct}, solution resistance (R_s), Warburg impedance (W) and constant phase element. The achieved R_{ct} values are 718.4 Ω and 307.6 Ω for BCPE and P(VLN)MCPE respectively. These results show that the modification of Poly(VLN) improves the sensing ability of the material (CPE) with high conductivity and less R_{ct}.

3.4. Active surface area of BCPE and P(VLN)MCPE

The electrochemical active surface area of BCPE (curve-b) and P(VLN)MCPE (curve-a) was studied using 1 mM K₄[Fe(CN)₆] in 0.1 M KCl using the CV method (Fig. 4). Here, the P(VLN)MCPE shows enhanced electrocatalytic activities with developed highest currents than BCPE. This statistic highlights the importance of the electrochemical polymerized electrode. This improved electrochemical activity is due to the larger surface area and extra active spots on the surface of the P(VLN)MCPE. The active surface areas of BCPE and P(VLN)MCPE were calculated using the Randles-Sevcik relation shown in Eq. (1) [24],

$$I_{\rm p} = 2.69 \times 10^5 \,{\rm n}^{3/2} \,{\rm A} \,{\rm D}^{1/2} \,{\rm v}^{1/2} \,{\rm C} \tag{Eq. 1}$$

Here 'n' represents contributing electrons in the oxidation-reduction response of $K_4[Fe(CN)_6]$, 'A' is the surface area of the working electrode dignified in cm², 'D' denotes the diffusion coefficient of $K_4[Fe(CN)_6]$ calculated in cm²/s, 'v' signifies the scan rate calculated in V/s, and 'C' for the $K_4[Fe(CN)_6]$ concentration measured in mM. The estimated electro-active surface area of BCPE and P(VLN)MCPE is found 0.036 cm², and 0.049 cm², respectively.

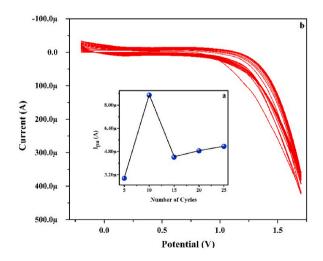


Fig. 1. (a) Plot a different of CV cycles vs I_{pa}. (b) CVs of 0.1 M poly (valine) on CPE with 10 electro-polymerized cycles at the scan rate 0.1 V/s.

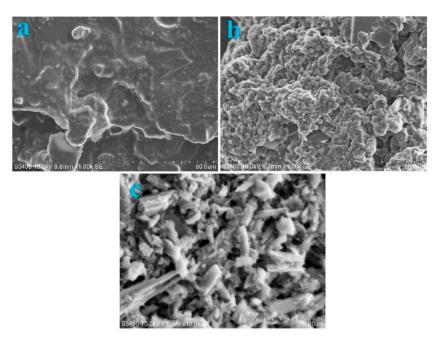


Fig. 2. SEM Pictures of (a) BCPE, (b) P(VLN)MCPE, and (c) after IGN reaction at P(VLN)MCPE.

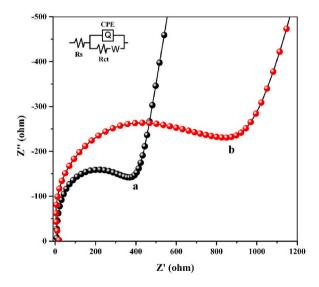


Fig. 3. Nyquists plots obtained for (a) P(VLN)MCPE and (b) BCPE.

3.5. Electrochemical behaviour of IGN

The electrochemical activities of established electrodes, BCPE (peak-a) and P(VLN)MCPE (peak-c)) were examined by studying the oxidation-reduction reaction of 1 mM IGN in 0.2 M PBS with pH 6.5 and also in a blank (curve-b) only 0.2 M PBS with 6.5 pH, using CV technique at 0.1 V/s scan rate and the potential gap of -0.25 to 0.65 V (Fig. 5). The enhanced oxidation-reduction peaks of IGN were seen at the P(VLN)MCPE (I_{pa} is 8.69 μ A at the E_{pa} of 0.361V) surface as compared to the BCPE (I_{pa} is 1.039 μ A at the E_{pa} of 0.0358V). These outstanding results are due to the electropolymerized VLN with enhanced electrocatalytic active surface area, more active spots, and good electrical contact among IGN, P(VLN), and CPE surfaces. Correspondingly, blank (only PBS) at the surface of P(VLN)MCPE does not provide any response. This result shows that the high electrochemical effect is only due to the presence of IGN on the surface of the modified electrode.

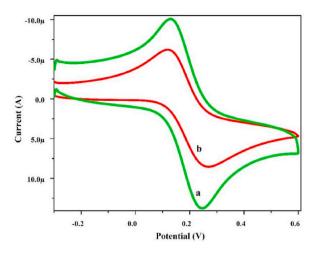


Fig. 4. Images of CVs, K4([Fe(CN)6] in 1mM and KCl 0.1 M at P(VLN)MCPE (peak-a) and BCPE (peak-b).

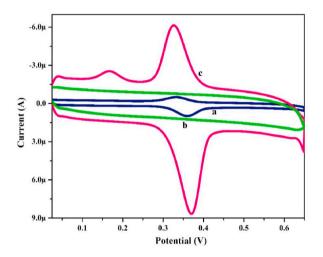


Fig. 5. CVs for 1 mM of IGN at BCPE (curve-a), for blank (curve-b) on P(VLN)MCPE, and P(VLN)MCPE (curve-c).

3.6. Analysis of pH effect

The effect of pH on the IGN activity (1 mM) at P(VLN)MCPE was checked by varying pH from 6.0 to 8.0, and the CVs of the corresponding solution were recorded at the scan rate of 0.1 V/s (Fig. 6a). From this study, we analyzed that as pH changes from 6.0 to 8.0, the electrochemical oxidation of signal peak potential moves to the negative direction, and the peak current is maximum at pH 6.5 (Fig. 6b and c). So, pH 6.5 was selected as the finest among all the pH, the analysis of this study demonstrates outstanding electron transfer and a strong electrochemical contact between the IGN and the electrode surface. Also, the pH v/s E_{pa} graphs show respectable linearity, and the equivalent relation is $E_{pa} = 0.715-0.052$ pH (V/pH) ($R^2 = 0.978$). The obtained slope value (0.052 V/pH) is near the theoretic value of 0.059. Hence, an equal number of protons and electrons are involved in the oxidation-reduction response of IGN.

3.7. Effect of scan rate

The effect of scan rate on the electrochemical detection of IGN in 0.2 M PBS with pH 6.5 at P(VLN)MCPE was studied using the CV method with changing scan rates varying from 0.025 to 0.375 V/s within the potential range from -0.25 to 0.65 V shown in Fig. 7a here both the peak currents (I_{pa} and I_{pc}) and peak potentials (E_{pa} and E_{pc}) is dependent of scan rate. As a result, with the increase of scan rate, the oxidation-reduction peak current also rises, the potential signals of the anodic peak show a shift toward the positive direction, and the potential of the cathodic peak signal shifts toward the negative direction. The graphs are plotted log v v/s log I_{pa} in (Fig. 7b) and v v/s I_{pa} (Fig. 7c) provides respectable linear relation and the equivalent relation are log (I_{pa}, A) = -4.157 + 0.849 log (v, V/s) (R² = 0.999) and I_{pa} (A) = 1.849 + 7.811 × 10⁻⁵ v (V/s) (R² = 0.998). At present the value of slope (0.849) of the following graph log v v/s log I_{pa} and the value of linear reversion coefficient (R² = 0.998) of the plot of v v/s I_{pa} values are closer to the theoretic value of 1,

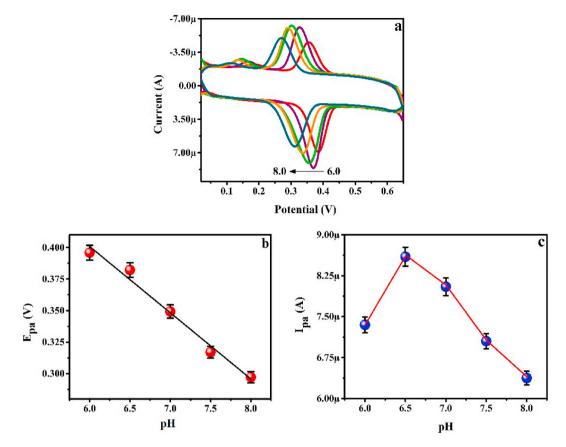


Fig. 6. (a) Images of CVs in 1 mM IGN at different pH ranging from 6.0 to 8.0 of 0.2 M PBS at 0.1 V/s scan rate. (b) Plot of E_{pa} vs. pH. (c) Plot of I_{pa} vs. pH.

signifying the response of oxidation-reduction reaction of IGN at the P(VLN)MCPE surface and the adsorption-controlled route is common their pathway. E_{pa} (V) = 0.445–0.076 log (v, V/s) (R² = 0.994) and E_{pc} (V) = 0.280–0.0554 log (v, V/s) (R² = 0.992) are the comparable linear relationship seen in Fig. 7d for the graph of log v v/s E_{pa} . Therefore, using Laviron's relation in this regard, the following relations (Eq. (2) and Eq. (3)) are utilized to determine the total number of electrons which contributed to IGN oxidation-reduction response [24],

$$B_{pa} = \frac{2.303 \text{RT}}{(1-\alpha)\text{nF}}$$
(Eq. 2)
2.303 × R × T

$$B_{pc} = \frac{2.303 \times R \times T}{\alpha n F}$$
(Eq. 3)

Here, n is the number of contributed electrons in the redox response of IGN, α is the charge transfer coefficient, F is Faraday's constant, T is the temperature, and R is the universal gas constant. B_{pa} and B_{pc} are the slopes of log v v/s B_{pa} and B_{pc} graph, respectively. In the redox response of IGN at the P(VLN)MCPE surface. The calculated value of the total number of transferred electrons is 2.01 (around 2) Scheme 1 shows the IGN probable oxidation-reduction reaction. Additionally, using the equation below (Eq. (4)), the surface coverage concentration (Γ) of IGN at P(VLN)MCPE was calculated.

$$\Gamma = I_{pa} \left[\frac{\theta}{nFA} \right]$$
(Eq. 4)

The concentration of IGN on the surface of P(VLN)MCPE was determined to be 1.1390 nM/cm².

3.8. Concentration variation of IGN

The capability of detection concerning LOD and LOQ of the P(VLN)MCPE was analyzed by changing the concentration of IGN was started ranging in 0. 2 μ M–5.0 μ M in 0.2 M PBS with 6.5 pH using the DPV technique and best tentative conditions (Fig. 8a). The DPV for changed concentrations of IGN shows that the absorption of IGN rises and the signal current of IGN also rises. After the

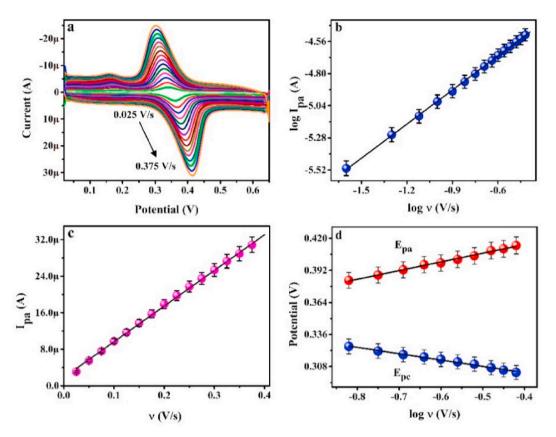
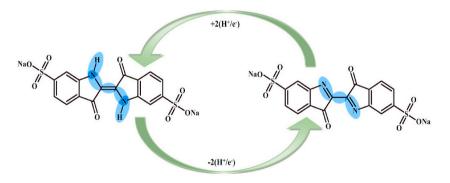


Fig. 7. (a) Images of CVs, 1 mM IGN on better pH with scan rates changed from (0.025 V/s to 0.375 V/s). Plotted graphs of, (b) log I_{pa} vs. log v. (c) I_{pa} vs. v. and (d) Epa vs. log v.



Scheme 1. Possible redux reaction of IGN.

experimental data, a diagram is plotted between the concentration of IGN and the signal current of IGN (Fig. 8b), from a good linear equivalence is spotted in the range from 0.2 to 5.0 μ M. The equivalent relation is I_{pa} (A) = 1.1499 × 10⁻⁶ + 2.103 (M) (R² = 0.994). The LOD and LOQ for IGN detection at P(VLN)MCPE were determined based on the slope of the calibration plot (2.103) and the standard deviation of the blank for five successive cycles. The determined values of LOD 0.0069 μ M and LOQ are found to be 0.023 μ M, respectively. The obtained LOD and the IGN-modified electrode sensor are equated with the earlier works and conforming data are presented in Table 1 [25–31]. This assessment study displays that the organized IGN modified sensor and technique provide suitable LOD for the analysis of IGN.

3.9. Repeatability, reproducibility, and stability of P(VLN)MCPE

The repeatability, reproducibility, and stability of the projected electropolymerized sensor (P(VLN)MCPE) were studied by the CV

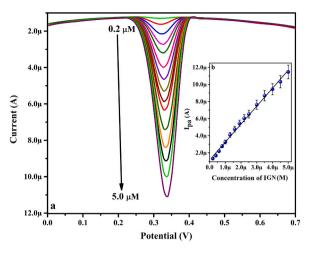


Fig. 8. (a) Differential pulse voltammetry for IGN with concentration changed from 0.2 μ M to 5.0 μ M in 0.2 M PBS at P(VLN)MCPE. (b) Plot of I_{pa} vs. [IGN].

Table 1

Assessments of present LOD value in dissimilar sensors and methods used in earlier Published IGN papers.

Techniques	Modified Electrodes	Linear range (µM)	LOD (µM)	Reference
CV	Poly (glycine) modified carbon paste electrode	2.0-60.0	0.11	[25]
Spectrophotometric	-	1.7-39.0	0.25	[26]
Resonance Rayleigh scattering	_	2.0-32.0	0.24	[27]
Square-wave voltammetry	Cathodically pre-treated boron-doped diamond	0.5-84.1	0.058	[28]
Surface enhanced resonance Raman spectroscopy	Silver colloids	1.0-0.1	36.50	[29]
DPV	SPCE	0.2–20	0.17	[30]
CV	SPCE	0.5-100	0.19	[30]
CV	SPE	0.5-100	0.20	[31]
DPV	P(VLN)MCPE	0.2-5.0	0.0069	Present work

method for the oxidation-reduction reaction of IGN in 0.2 M PBS with 6.5 pH at the scan rate of 0.1 V/s. Duplicability was confirmed with 5 succeeding CV cycles for the always fixed analyte of IGN at the surface of P(VLN)MCPE, here electrode was different at the finish of the respective cycle. The calculated comparative standard deviation value is about 1.025 %, which proposes that the modified electrode has decent reproducibility. The repeatability was tested based on 5 succeeding CV cycles for the IGN here using different analyte ends of individual cycles at the surface of P(VLN)MCPE always fixed. The determined relative standard deviation value is about 0.544%, which shows decent repeatability of the modified sensor. Also, the stability of P(VLN)MCPE was examined by recording the CV for IGN at optimized conditions. Here, the modified sensor was stored in a sealed container at a laboratory temperature of 25 °C for

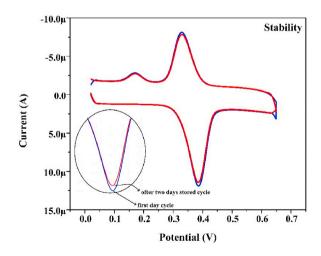


Fig. 9. CVs for IGN at the surface of P(VLN)MCPE with respect to 1st day and 3rd day electrode storage.

two days, after that the same electrode was used for the detection of IGN (Fig. 9). Also, the IGN response was tested at the surface of P (VLN)MCPE in the 1st day (Fig. 9). The outcomes show that only 3.71 % of IGN peak current was declined as compared to the initial peak current of IGN. This outcome shows decent stability of the prepared sensor.

3.10. Interference study

The planned electrode for the detection of IGN was studied in different metal ions and organic compounds in 0.2 M PBS with 6.5 pH at the scan rate of 0.1 V/s and the results of the outcomes are displayed in Fig. 10. The detection of 1 mM IGN was done in some metal ions like Mg^{2+} , Fe^{2+} , K^+ , Zn^{2+} , and some organic molecules are also presented (1.0 mM) like alanine (AN), methyl orange (MO), riboflavin (RN), tartrazine (TZn), sucrose (SUs), and titan yellow (TNY) and we observed a less significant effect of these molecules on the redox action of IGN. The relative inaccuracy for the assessment of IGN in the presence of interferents is achieved at less than ± 5 %, this simplifies that the prepared electrode has good, suitable and it is interference-free.

3.11. Analysis of water sample

The applicability of P(VLN)MCPE was investigated for the determination of IGN compounds in a water sample. In this study, IGN analysis was conducted on a tap water sample using a spike recovery technique. The P(VLN)MCPE exhibited good recovery rates for IGN in the water sample, ranging from 95.31 % to 100.24 %, the results of these outcomes are presented in Table 2.

4. Conclusions

The present study is the simplest, most affordable, and low-priced electrochemical sensor P(VLN)MCPE was prepared resourcefully using a green process for the selective and sensitive determination of IGN. The CPE surface was stimulated by evolving an active film of P(VLN) done a modest electro-polymerization method. The improved electrocatalytic surface zone of P(VLN)MCPE exhibits a quicker electron transfer throughout the IGN oxidation-reduction response with high electrochemical catalytic movement. The surface characters of P(VLN)MCPE and BCPE were established positively through CV, SEM, and EIS methods. The P(VLN)MCPE surrounds an enhanced electrocatalytic activity with a decent linear association, lesser LOD, respectable stability, reproducibility, and good repeatability near the oxidation-reduction reaction of IGN. Also, the P(VLN)MCPE was used for real sample analysis of IGN recovery in a tap water sample in the range of 95.31 %-to 100.24 %.

Data availability statement

Data will be made available on request.

Ethical approval

This article is original, has not been published previously in any form or language and this article is not under consideration for publication elsewhere.

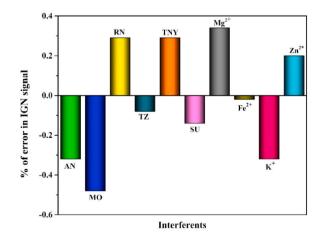


Fig. 10. Graph of interferents vs % of sample error in IGN peak potential for metal ions and organic compound in the presence of IGN.

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Table 2					
Recovery	data	of IGN	l in	water	sample.

Sample	Added (µM)	Found (µM)	Recovery (%)
	0.8	0.801	100.24
Water	1.3	1.264	97.24
	1.6	1.525	95.31
	2.2	2.174	98.80

CRediT authorship contribution statement

Kanthappa Bhimaraya: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. Jamballi G. Manjunatha: Conceptualization, Data curation, Formal analysis, Resources, Supervision, Validation, Visualization, Writing – review & editing. Hareesha Nagarajappa: Data curation, Formal analysis, Visualization, Writing – review & editing. Ammar M. Tighezza: Conceptualization, Data curation, Visualization. Munirah D. Albaqami: Data curation, Formal analysis, Visualization. Mika Sillanpää: Data curation, Visualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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K. Bhimaraya et al.

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