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RESEARCH ARTICLE

ELECTROCHEMICAL SENSOR FOR THE DETERMINATION OF NICOTINIC ACID ON GLASSY CARBON ELECTRODE

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Abstract

A differential pulse voltammetric (DPV) method for the sensitive determination of nicotinic acid (NA) using a glassy carbon electrode (GCE) is presented in this study. Nicotinic acid, a crucial biomolecule involved in various metabolic pathways, requires accurate and reliable analytical methods for its quantification. The proposed method employs a GCE, which showed to be the best electrode for the determination of nicotinic acid. The voltammetric behavior of nicotinic acid was investigated in a suitable supporting electrolyte and the effects of various experimental parameters such as scan rate, pulse amplitude, pulse frequency, pH were optimized. The method demonstrated a high level of sensitivity with a low detection limit, achieving precise quantification of nicotinic acid in complex samples. The optimized method exhibited a dynamic concentration linear range of 2.5×10^{-8} - 8.0×10^{-3} molL⁻¹ with a detection limit of 3.28×10^{-9} molL⁻¹. The developed DPV technique provides a robust and efficient approach for the determination of nicotinic acid in pharmaceutical samples offering potential applications in biochemical research and clinical diagnostics.

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Introduction:-

Nicotinic acid (NA), commonly known as niacin or vitamin B3, is an essential water-soluble vitamin crucial for various physiological functions. Nicotinic acid is an organic compound with the molecular formula pyridine-3-carboxylic acid (C₆H₅NO₂). It is a derivative of pyridine, with a carboxyl group (COOH) at the 3-position [1]. As a member of the B vitamin family, nicotinic acid plays a pivotal role in metabolism by facilitating the conversion of carbohydrates, fats, and proteins into energy. It is a precursor to coenzymes, specifically nicotinamide adenine dinucleotide (NAD) and its phosphorylated form (NADP), which are vital for numerous enzymatic reactions within the cell. Nicotinic acid plays important biological roles in the human body. It enters the body through eating foods and fruits (including yeast, fish, milk, eggs, green vegetables and cereal grains) and also is produced in the body [2-3]. The biological significance of nicotinic acid extends to its involvement in maintaining healthy skin, supporting the nervous system, and ensuring proper digestive function. Deficiency in nicotinic acid can lead to a condition known as pellagra, characterized by dermatitis, diarrhea, and dementia. Given its importance, it is crucial to monitor and maintain adequate levels of nicotinic acid through diet or supplementation [4]. NA is crucial for numerous biological processes. It functions as a precursor to coenzymes NAD and NADP, which are integral to energy metabolism and various biochemical reactions. Given its importance in human health, accurate

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measurement of nicotinic acid levels is essential for nutritional assessments and therapeutic monitoring. In addition to its nutritional roles, nicotinic acid has been recognized for its pharmacological properties. It has been used therapeutically to manage dyslipidaemia, as it can effectively lower cholesterol levels and triglycerides in the blood, thereby reducing the risk of cardiovascular diseases [5].

Various analytical methods such as chromatographic and spectrophotometric techniques are used for the determination of NA. High performance liquid chromatography (HPLC) and capillary electrophoresis, gas chromatography-mass spectrometry (GC-MS), flow injection spectrophotometry, micellar electrokinetic capillary chromatography and Fluorimetry have been used for the determination of NA [6-13]. These methods need to use complex instruments. Therefore, there is a growing need for appropriate sensing systems capable of sensitive, rapid and low cost determination of NA. Due to its diverse applications and critical physiological roles, accurate and reliable quantification of nicotinic acid is essential for both clinical and research purposes. Electrochemical methods, particularly voltammetry, offer advantages in terms of sensitivity, specificity, and rapid analysis. This study explores the use of differential pulse voltammetry (DPV) with a glassy carbon electrode (GCE) for the precise determination of nicotinic acid, aiming to provide a robust analytical tool for monitoring and research applications [14-15].

Differential Pulse Voltammetry (DPV) is a highly sensitive electrochemical technique widely used for the analysis of various analytes, including pharmaceuticals, biomolecules, and environmental contaminants. Its application in the determination of nicotinic acid (NA) provides a valuable tool for precise and reliable quantification of this essential compound [16]. Differential Pulse Voltammetry (DPV) stands out due to its high sensitivity and selectivity, making it well-suited for detecting low concentrations of nicotinic acid. In DPV, a series of voltage pulses are superimposed on a linear scan of the potential, and the resulting current responses are recorded. This technique enhances the resolution of redox processes and reduces background noise, allowing for the precise determination of analytes even at trace levels. This study shows that GCE provides a powerful approach for the precise determination of nicotinic acid, facilitating its monitoring and ensuring effective utilization in both clinical and research settings for determination of nicotinic acid in pharmaceutical samples.

Material and Methods:-

Chemicals:-

All chemicals were of Analytical grade and were used as received without further purification. Nicotinic Acid, Boric Acid, o-Phosphoric Acid, Glacial Acetic Acid, NaOH, Conc. HCl, Sodium Citrate, Citric Acid anhydrous, Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate, Sodium acetate, Acetone. Double distilled water was used for the preparation of aqueous solutions having a specific conductivity 0.4 -0.9 μS

Methods:-

Preparation of Standard nicotinic acid Solution (1×10^{-4}): Nicotinic Acid: 250ml Standard solution was prepared by dissolving 0.0003g of nicotinic acid in distilled water as a stock solution.

Preparation of Buffer Solution:-

1. BR Buffer (0.04M) : Prepare buffer solution by adding 4.948g boric acid, 4.56ml glacial acetic acid and 5.84ml of o-phosphoric acid in distilled water
2. Citric acid-sodium citrate buffer: Prepare 0.1M buffer solution in 1litre by adding 24.27g sodium citrate and 3.358g of citric acid anhydrous solution in distilled water
3. Phosphate Buffer: Prepare buffer solution in 500ml by adding 14.1g disodium hydrogen phosphate and 11.45g of potassium dihydrogen phosphate.
4. Acetate Buffer: prepare 1litre by adding 7.72g sodium acetate and 0.352ml acetic acid.

Preparation of different pH solution of BR buffer from BR buffer of pH 2:-

Take 100ml buffer solution and then adjust at different pH from 2 to 9 with NaOH or HCl. Dilute 10ml standard solution with adjusted pH solution.

Preparation of Different concentration solution:-

Perform serial dilutions of the stock solution to prepare lower concentration solutions from $1 \times 10^{-4}\text{M}$ with citrate buffer solution for concentration study and for the determination of nicotinic acid in real samples.

Instrumentation:-

All voltammetric measurements study has been performed on PhadkeSTAT 20 potentiostat. A three electrode system employing an Ag/AgCl (3M KCl) as reference electrode, platinum electrode as counter electrode and glassy carbon as working electrode was used. The pH measurements were performed using an ELICO LI 120 pH meter.

Determination of Nicotinic acid:-

Differential pulse voltammetric (DPV) studies were carried out with appropriate quantity of the analyte (NA) in 50mL standard volumetric flask and then making up to the mark with pH 6.0 Citrate buffer (Cit).The solution was then transferred into an electrochemical cell and the measurements were carried out at $25 \pm 0.2^\circ\text{C}$. N_2 gas purging was not required as oxygen did not interfere in the measurements. DPVs were recorded within the potential range -0.1 to -1.2 V with a scan rate of 50 mVs^{-1} and modulation amplitude of 50 mV.

Results and Discussion:-**Effect of pH:-**

The effect of change in pH on peak potential for NA was investigated by different pulse voltammetry from pH 2 to 9 employing Britton-Robinson (BR) buffer (0.04M) by DPV.. Standard solution of NA ($1 \times 10^{-5}\text{M}$) was used to find the optimum pH of the supporting electrolyte at GCE. Fig. 1 represents the graph of I_p vs E_p for various pH of BR buffer . The plot of E_{pvs} pH shows a negative shift of E_p values (Fig 2) with increasing pH suggest the involvement of protons for the electro-reduction of NA with the involvement of proton transfer preceding the potential determining step [17]. The peak currents were found to increase in the beginning with the increase in pH (Fig 2) showing maximum at pH 6.0 and decrease thereafter. This could be due to the fact that the reduction became kinetically less favorable due to repulsive electrostatic interactions with the surface of the electrode. Therefore this pH (pH 6) was selected as the optimum pH for further studies

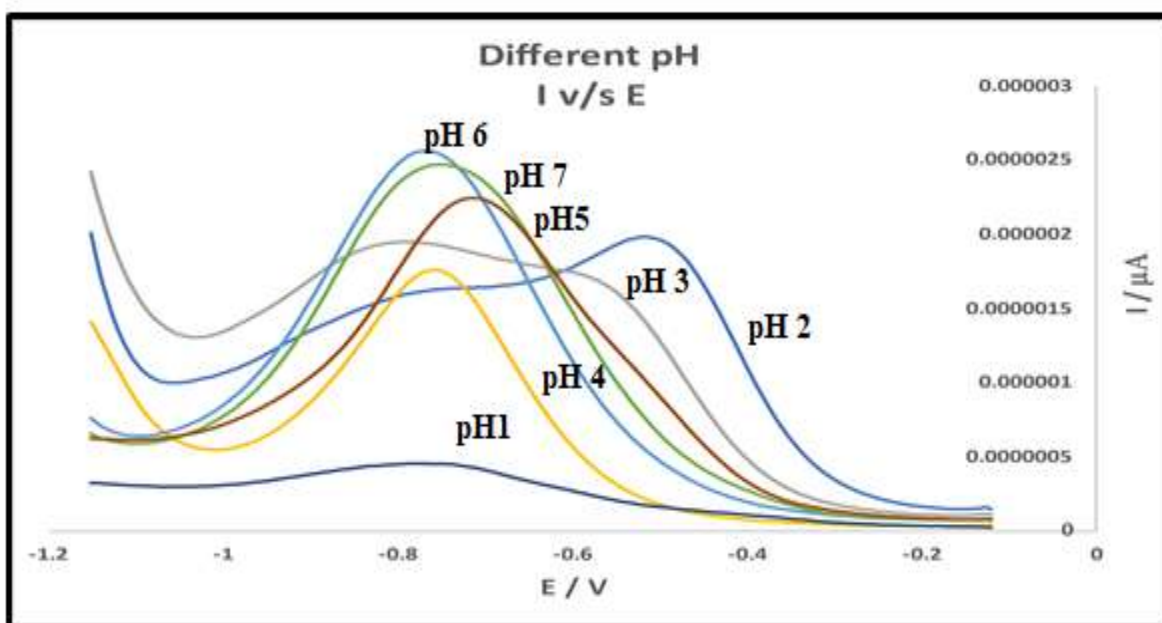


Fig. 1: pH study by Differential pulse voltammetry for reduction of $1 \times 10^{-5}\text{M}$ NA at; glassy carbon vs. Ag/AgCl; in 0.04M BR buffer ; scan rate 100mV/s at 25°C

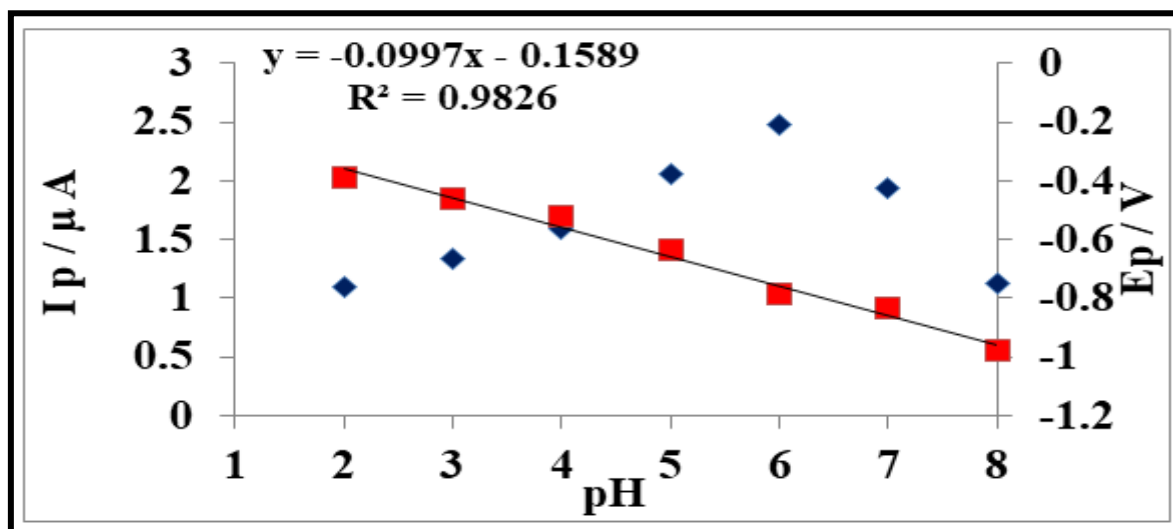


Fig. 2: Plot of E_{pvs} pH and I_{pvs} pH by Differential pulse voltammetry for reduction of 1×10^{-5} M NA at; glassy carbon vs. Ag/AgCl; in 0.04M BR buffer ; scan rate 100mV/s at 25°C.

Effect of supporting electrolyte:-

The effects of several supporting electrolytes viz. phosphate buffer, acetate buffer, citrate buffer, BR buffer at pH 6 for 1×10^{-5} M NA on peak current was tested in Fig (3). The concentration of the buffers was taken as 0.1M except for BR buffer where concentration was 0.04M. Amongst all the buffers used, Citrate buffer gave the best response in terms of peak current and peak shape for NA. Thus Citrate buffer was chosen for further experiments. Further optimization of buffer concentrations was carried out by varying citric acid- sodium citrate concentration in the range from 0.05M, 0.1M, 0.15M and 0.2M the best peak response was observed for 0.1M citric acid- sodium citrate (pH 6) and hence was used for the further studies.

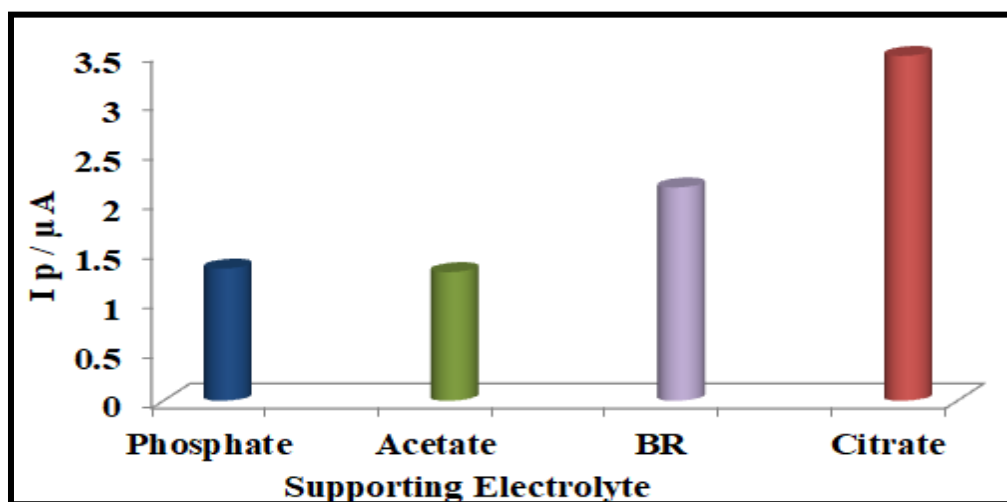


Fig. 3: Plot of I_{pvs} supporting electrolyte by Differential pulse voltammetry for 1×10^{-5} M NA at; glassy carbon vs. Ag/AgCl; scan rate 100mV/s at 25°C.

Determination of NA by Differential Pulse Voltammetry (DPV):-

Effect of pulse time:-

The effect of pulse time were studied for 1×10^{-5} M for the purpose of investigating their reaction mechanism which are shown in Fig (4). The influence of pulse time was studied from 0.2 to 0.4 secs for the NA at GCE in citric acid- sodium citrate (pH 6). the peak current varied linearly with the increase in the pulse time for NA.

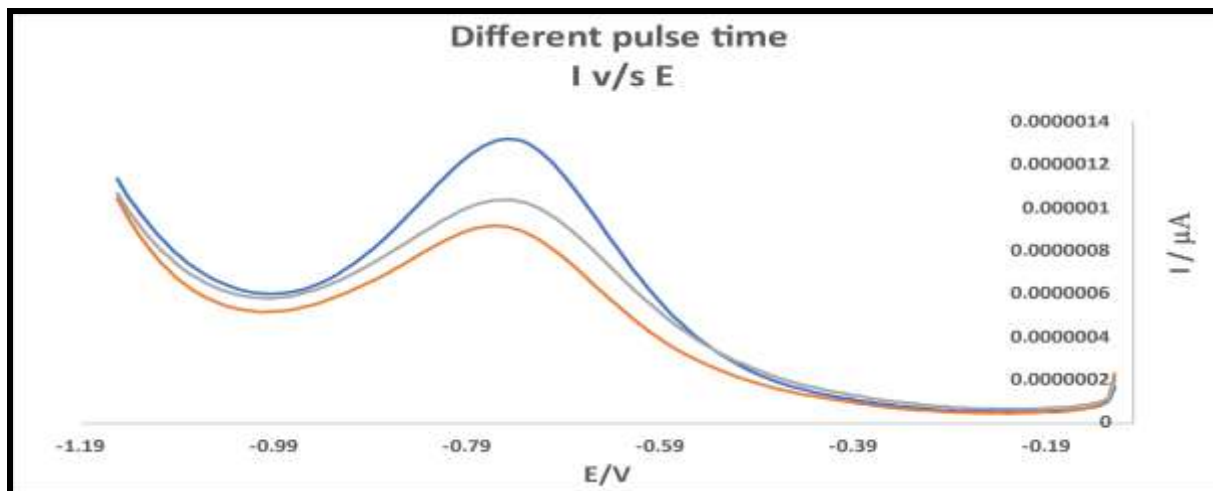


Fig: 4 Voltammogram of NA at pulse time 0.1sec (■), 0.2sec (■) and 0.3sec (■) at 1×10^{-5} MNA in 0.1M Citrate buffer ; at; glassy carbon vs. Ag/AgCl; scan rate 100mV/s at 25⁰C.

Effect of pulse size:-

The effects of pulse size were studied for 1×10^{-5} MNA for the purpose of investigating their reaction mechanism which is shown in Fig (5). The influence of pulse size was studied from 100 to 1000V for the NA at GCE in citric acid- sodium citrate (pH 6) . The anodic and cathodic peak current were independent with variations in potential (100V to 1000V) and time (0.2 to 0.4secs) for 1×10^{-5} M NA reconfirming that the process of NA reaching the GCE surface was purely by diffusion.

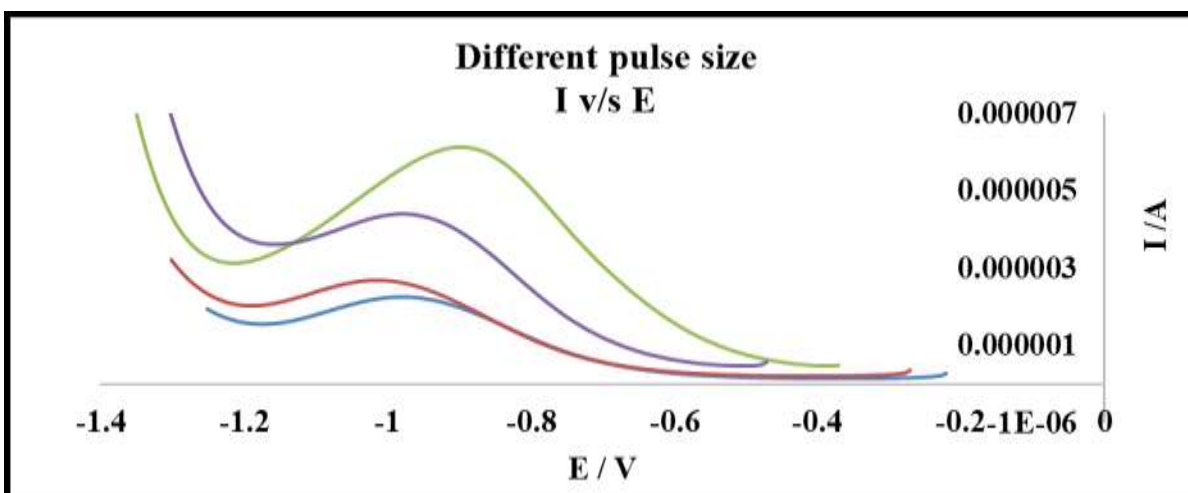


Fig: 5 Voltammogram of NA at pulse size 20mV (■), 30mV (■), 40mV (■) and 50mV (■) at pH=6 in 0.1M Citrate buffer ; at; glassy carbon vs. Ag/AgCl; scan rate 100mV/s at 25⁰C

Effect of concentration:-

The DPV technique was used for determination of NA at GCE the optimum conduction of instrumental variables where pulse size 50mV, pulse time 0.1sec, current range 200μA. the linear working range (LWR), empirical limit of detection (LOD) and correlation coefficient were determined and are presented in Table 1. Fig. 6 is Plot of NA at glassy carbon electrode in 0.1M citrate buffer (pH 6.0)

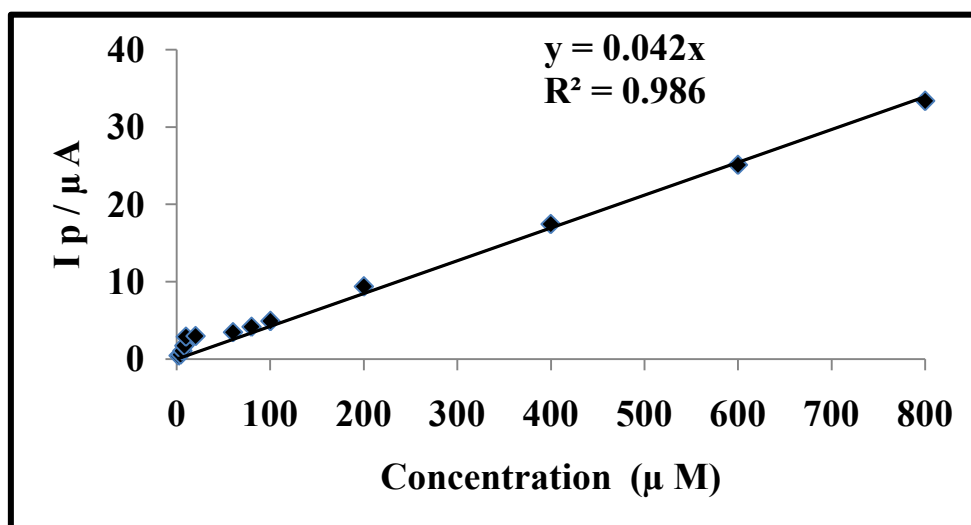


Fig. 6 Plot of I_p vs Concentration (μM) of NA at glassy carbon electrode in 0.1M citrate buffer (pH 6.0) at; glassy carbon vs. Ag/AgCl;; scan rate 100mV/s , 0.1sec, 50mV at 25^oC

Validation studies, interference studies and analytical applications:-

For validation of the proposed method, various parameters such as repeatability, reproducibility, precision and accuracy of the analysis were obtained by performing five replicate measurements of $1 \times 10^{-5}\text{M}$ NA over intraday assay (single day, $n = 5$) and inter-day assay (for a period of 1 week). Satisfactory mean percentage recoveries (%R) and relative standard deviations (% RSD) were obtained and are presented in Table 2. The recoveries obtained confirmed high precision and accuracy of the proposed method. In order to further extend the validity of the proposed method, verification of the matrix effect on NA on determinations by DPV was studied. The influence on the peak heights of some interferents commonly present, some of them which form the major components of multivitamin pharmaceutical preparations were evaluated.

Table 1: Analytical parameters for electrochemical determination of NA at glassy carbon electrode in 0.1M

Molecule	LOD	%RSD	LWR	LRE	r
Statistical data for individual molecule					
NA	$3.28 \times 10^{-9}\text{M}$	1.72	2.5×10^{-8} to 8.0×10^{-3}	$I_p (\mu\text{A}) = 0.0424(\mu\text{M})$	0.9869

citrate buffer (pH 6.0) at; glassy carbon vs. Ag/AgCl;; scan rate 100mV/s , 0.1sec, 50mV at 25^oC

Table 2: Precision and Bias of assay for standard NA solution by DPV (n =5)

Molecule	Concentration taken (mol L^{-1})	Mean found concentration (mol L^{-1})	Mean recovery %	Bias %	Precision % RSD
HIS	Intra day				
	1×10^{-5}	$0.97 \times 10^{-5}\text{M}$	98.4	0.52	1.52
	Inter day				
	1×10^{-5}	$1.09 \times 10^{-5}\text{M}$	100.9	- 0.35	2.1

The tolerance limit for interfering species was considered as the maximum concentration that gave a relative error in terms of ΔI_p less than $\pm 5.0\%$ at a concentration of $1 \times 10^{-5}\text{M}$ NA. Five replicates of each experimental set were performed. The results showed tolerance limit of 150 fold of ascorbic acid, 100 fold for citric acid and thiamine hydrochloride, 50 fold for tartaric acid, 20 fold for riboflavin and 10 fold for cyanocobalamin showing that the present modified electrode was highly selective towards the determination of NA in the presence of common

physiological interferences. The validity of the G-CME electrode was verified in the determination of NA in various pharmaceutical preparations by standard addition method (Table 3)

Table 3: Assay of NA in pharmaceutical preparations (n =5)

Pharmaceutical preparation	Nicotinic acid	
	Amount of drug in the sample (mg)	Amount of drug obtained in the proposed method (mg) \pm RSD
Niaspan	500.0	497.9 \pm 1.1
Niacor	1000.0	998.5 \pm 2.2
Femcinol-A Gel	40mg	50.4 \pm 1.8

Conclusion:-

A glassy carbon electrode sensor was used for the detection and quantification of Nicotinic acid. An acceptable linear dynamic range and detection limit were obtained. The developed differential pulse voltammetric method was applied for the determination of Nicotinic acid in tablets and gel with good sensitivity and selectivity in the pharmaceutical dosage forms. The reliability and stability of the electrode offers possibility to be used in quality control laboratories for identification and quantification of real samples

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