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Novel Electrochemical Biosensor Based on Drop Nanosense Used for Estimated pH Level of SARS-COR2 Viral Transport Media Prepared in Basrah University /Iraq

Hisham Faiadh Mohammad[1,3*] and Hala Fadhel Hassan[2]

[1]*Biology Department, College of Science – University of Basrah*

[2]*Marine Biology Department, Marine Science Center – University of Basrah*

[3]*School of Pharmacy and Bioengineering, Keele University, U.K*

***Corresponding Author**
Hisham Faiadh Mohammad, Biology Department , College of Science - University of Basrah.

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Abstract

In this work, the electrochemical scan of viral transport media (VTM) on a screen printed gold electrode was studied using cyclic voltammetry technique. The dependence of the anodic and cathodic currents on VTM and screen printed gold electrodes was investigated to optimize the experimental conditions of pH values determination. The oxidation and reduction mechanism in this study were proposed. Under the optimum pH conditions, the oxidation and reduction peak current were linearly proportional to the pH value of VTM in the range from 6.5 to 9.0 with a standard behaviour of control sample as reference. The novel proposed method to optimum pH level determination of real VTM samples was successfully applied. Three pH conditions of viral transport media (VTM) were compared to the standard reference sample. The pH value of VTM was investigated using unique electrochemical sensing assay. All VTM were prepared in Basrah University-Faculty of Pharmacy Lab and the pH level was determined by pH meter within 24h and compared with DropSens technique at the same time. Overall, the pH value of VTM which identified rapidly was checked electrochemically within 10 second as a routine test. The electrochemical behaviour of VTM pH and its value was studied using (C.V) cyclic voltammetry carried out at a screen printed gold electrode which was a novel method. The oxidized and reductized reaction peak at the surface of screen printed gold electrode were determined the value of pH according to the specific electrochemical behaviour which depended. Both peaks on cyclic voltammograms corresponded to electrooxidation and electroreduction were used to detect pH level of VTM and which increase the current dramatically with pH value, respectively. The aim of the current study was to evaluate the performance characteristics of the electrochemical pH estimated sensing assay in comparison to conventual pH meter tool of VTM solution. These electron transformations resulted in increased C.V oxidation and reduction currents as recorded using a platinum working electrode it was suggested that the higher oxidation and reduction peak currents were due to an increase in the pH value of VTM solution.

Keywords: Electrochemical, SARS-COR2 VTM, Basrah-Iraq, pH VTM, C.V Estimation

1. Introduction

VTM is currently used as a suitable medium for the corona virus detection assay [1]. Severe and acute respiratory syndrome related coronavirus-2 infection needs accurate diagnostic testing to identify suspected patients infected with (SARS–COR2) which is considered the global pandemic of COVID-19 when occurred in the end of 2019 [2]. Covid-19 has become a global pandemic because of the rapid spread and imaginative numbers of cases and deaths. It becomes the most important issue that brings the attention of not only scientists but also the press, international and publishing houses, etc… In a few countries, the use of diagnostic testing on a massive scale has been a cornerstone of successful containment strategies. In contrast, the United States, hampered by limited testing capacity, has prioritized testing for specific groups of persons [3,4]. Real-time reverse transcriptase polymerase chain reaction–based assays performed in a laboratory on respiratory specimens are the reference standard for COVID-19 diagnostics [5]. However, point-of-care technologies and serologic immunoassays are rapidly emerging [6]. Although excellent tools exist for the diagnosis of symptomatic patients in well-equipped laboratories, important gaps remain in screening asymptomatic persons in the incubation phase, as well as in the accurate determination of live viral shedding during convalescence to inform decisions to end isolation. Many affluent countries have encountered challenges in test delivery and specimen collection that have inhibited rapid increases in testing capacity [7]. These challenges may be even greater in low-resource settings. Urgent clinical and public health needs currently drive an unprecedented global effort to increase testing capacity for SARS–COR2 infection [8].

A current need in diagnostic microbiology laboratories is for a rapid, molecular-based assay with high sensitivity and quick kit for detection of diseases [9]. Isothermal nucleic acid amplification allows nucleic acid amplification in a very narrow temperature range, eliminating the need for expensive thermal cyclers and allowing for results to be obtained very quickly [10]. The nucleic acid amplification test is a simple-to-use, automated test for viruses that is intended to provide the sensitivity of a molecular test with the quick results that a traditional rapid antigen test provides [11]. The rapid test can provide results within 15 min of initiating the test [12]. Specimen preparation, lysis, and nucleic acid amplification are all accomplished with minimal hands-on time [13]. Several laboratory methods are available for detecting viruses and aiding in the diagnosis of viral infections [14]. Commonly used methods in the lab include viral culture, direct fluorescent antibody (DFA) staining, immunochromatographic virus antigen detection-based assays, and nucleic acid amplification assays [15]. Unfortunately, these assays are laborious, subjective, technically demanding, and generally exhibit lower sensitivity when used alone rather than in conjunction with viral culture [16]. Real-time reverse transcription-PCR (RT-PCR) is becoming increasingly accepted as a gold standard for detection of infectious viruses, but it is technically demanding, laborious, and expensive; all these factors limit the usefulness of the technique in an outpatient setting. Antigen detection methods are available for rapid diagnosis of viral infections from respiratory specimens. These assays show benefit in Emergency Departments and outpatient settings due to their ease of use and rapid [17]. For these reasons, many clinical laboratories employ rapid antigen-based assays as their first-line diagnostic test for infectious virus infections [18]. Though generally exhibiting very high specificity and positive predictive values, the major limitations of currently available rapid antigen tests involve their low and widely variable sensitivity [19].

2. Experimental Methodology

2.1 Preparation of VTM Solutions

18 samples of the VTM solutions with different values of pH (6.5, 7.0, 7.5, 8.0, 8.5, and 9.0) were measured and which described according to Verano and Michalski protocol, and prepared in central lab / University of Basrah - Faculty of pharmacy (see Figure 1.), [21]. The solution of VTM samples were investigated by immersed them with SPGEs [22].

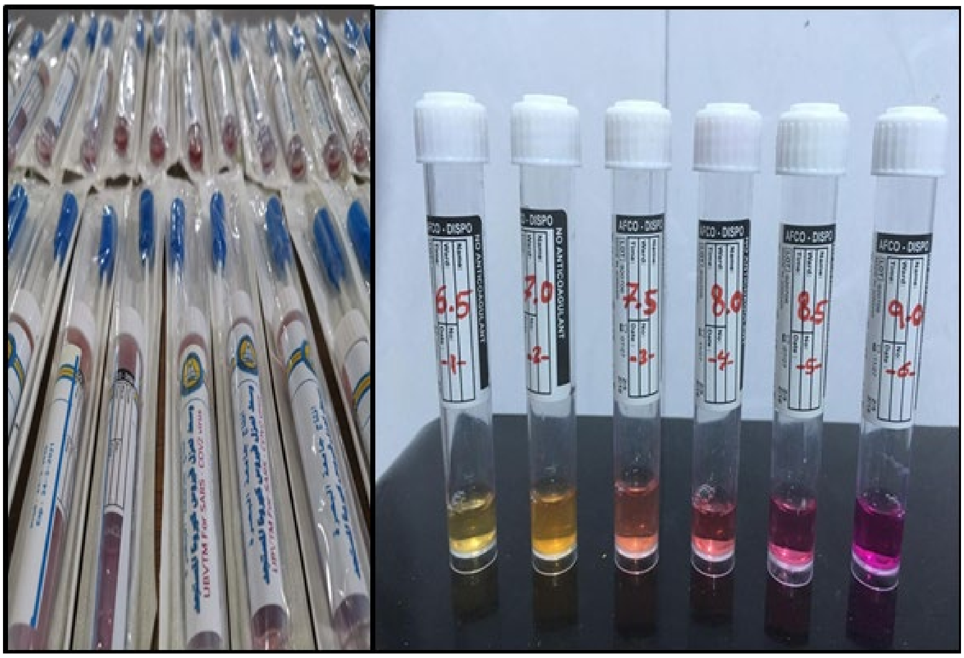

Figure 1: (A) VTM Solution Samples Prepared in University of Basrah (B) VTM Solution Samples in Different pH Values Measured by pH Meter

2.2 Electrochemical Experimental Measurements

All cyclic voltammogram (CV) of electrochemical measurements were carried out on a DropSTAT4000P potentiostat instrument (from DropSens-Metrohm) controlled by Autolab software using DropSens screen-printed gold electrodes (SPGEs); the voltage ranges from −0.5 to +0.5 V and the scan rate of 10 mV/s were used. SPGEs have a conventional three electrode configuration with gold working and counter electrodes and Ag/AgCl pseudo-reference electrode [23]. The CV measurements were carried on SPGEs with all samples of VTM which immersed in the surface of SPGEs, see Figure. 2(A-C) [24].

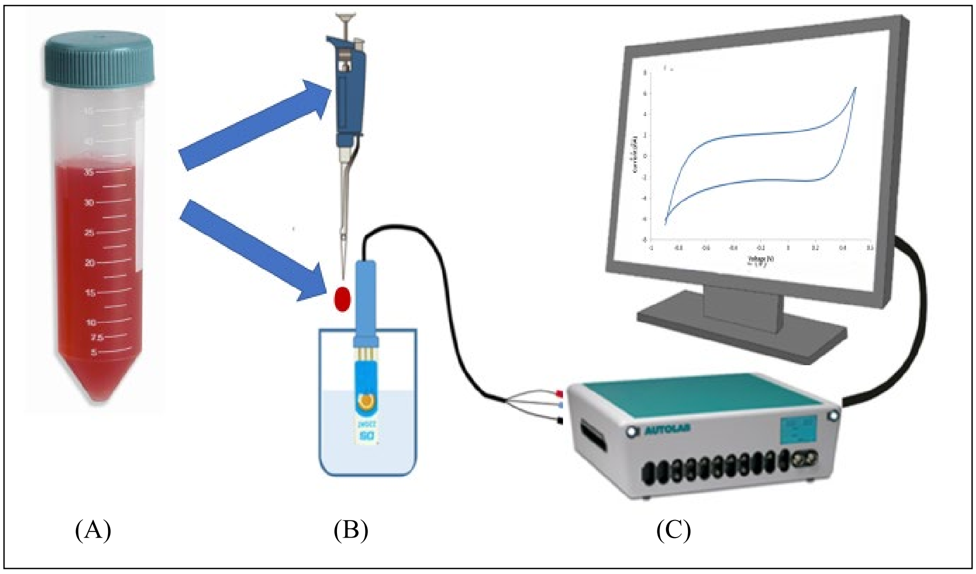

Figure 2: (A) VTM Solution Sample, (B) Drop Sens Screen Printed Gold Electrode, (C) Schematic Diagram of Drop Sens Three-Electrode Assembly Shows the Electrochemical Sensing Procedure Set-Up

3. Electrochemical Experimental Results

3.1 Cyclic Voltammogram Sensing Assay of VTM Solution

The electrochemical measurements of VTM samples were carried out using DropSens potentiostat and screen printed gold electrodes. Potential was recorded against Ag/AgCl reference electrode. Typical cyclic voltammograms for VTM in different values (i.e. from low pH to high) are shown sin Figure. 3.Generally, the CV graphs in Figure. 3 are almost featureless in the selected voltage range from−0.5 V to +0.5 V which was chosen deliberately in order to avoid electrochemical reactions on the surface of screen printed gold electrodes. Slight increase in the cathode current at −0.2 V indicates the beginning of hydrogen reduction. The values of cathodic current (Ic) at−0.5 V appear to increase with the increase in pH value (or with the largest current shown for highest level of VTM pH, While the lowest current appeared with the acidic level of pH solution. That means the reaction which carried on the surface of gold electrodes act as promoter increasing the current. These results are very important since we establish a correlation between the values of current and pH level in VTM solution. The results of these measurements in Figure. 3(A-F) show substantial increase in cathodic and anodic current of VTM sample (Figure. 3F) upon increasing the pH value, while this effect is much less pronounced for lowest pH value in VTM samples (Figure. 3A). In addition to that, the oxidation and reduction peaks appeared on CV curves for VTM samples in Figure. 4A which are definitely related to electrochemical reactions associated with the cathodic current (Ic) which increased also with pH level increasing. The anodic peak increases with the increase in pH value as well. The inset in Figure. 4B shows the dramatically increase of both anodic and cathodic current at ±0.5V and pH comparison. However, such electrochemical reactions do not appear on reference (control) sample in Figure. 5B. So, the oxidation and reduction peaks are most-likely related to the interaction of pH level with chemical reaction.

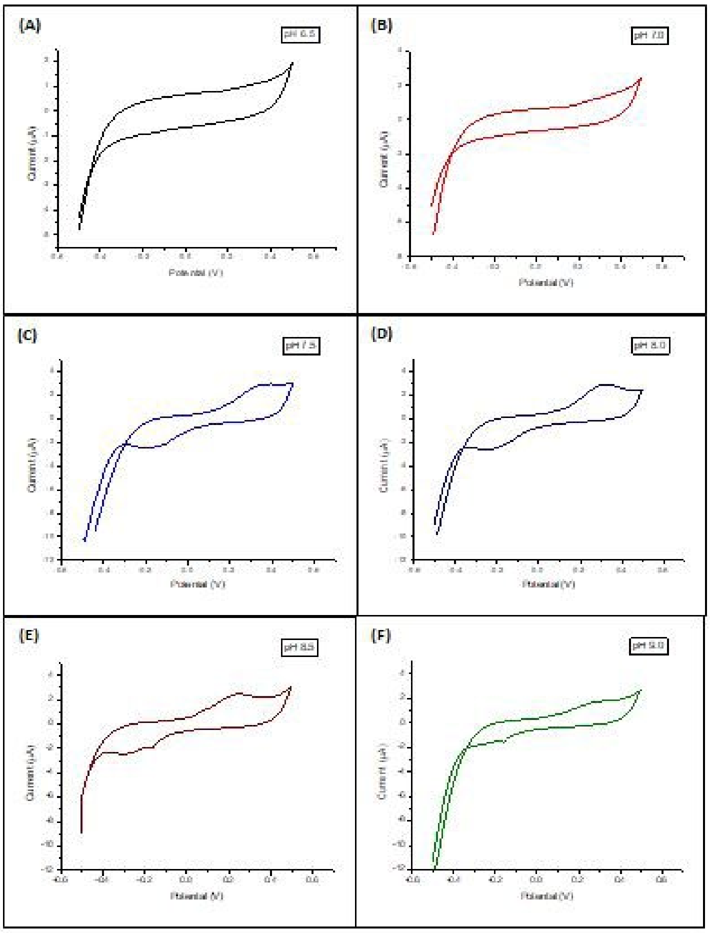

Figure 3: (A-F) Cyclic Voltammogram Recorded on Different Values of VTM pH from (6.5-9.0)

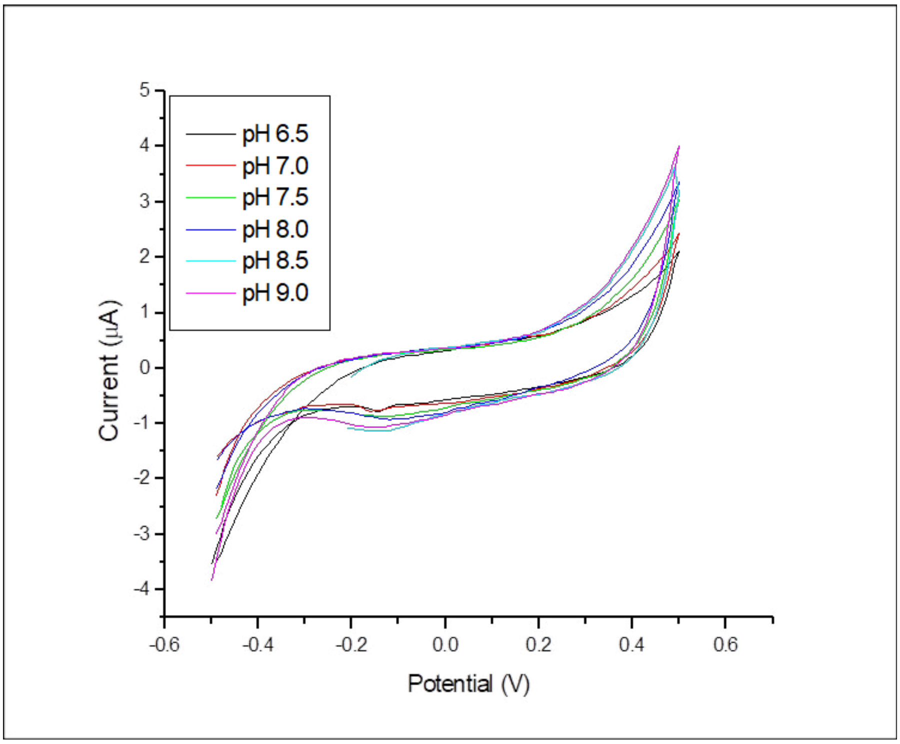

Figure 4: (A) Cyclic Voltammogram Recorded on Different Values of VTM, (A-F); CV Curves for Clear Reference Medium are Shown on the Graph. (B) The Inset of Anodic and Cathodic Current at ±0.5V and pH Comparison Shows the Dramatically Increase

3.2 The Relative Changes Study of VTM Solution Conductivity

To analyse the effect of pH value on CV characteristics of VTM samples, the effect of extra ions on conductivity of VTM due to the reaction has to be considered. In order to find out the true behaviour of pH value on electrode surface, the values of cathode current (Ic) of VTM samples has to be normalized by the reference current Iref of control sample which used such as a reference in particular value of pH (Figure. 5A).

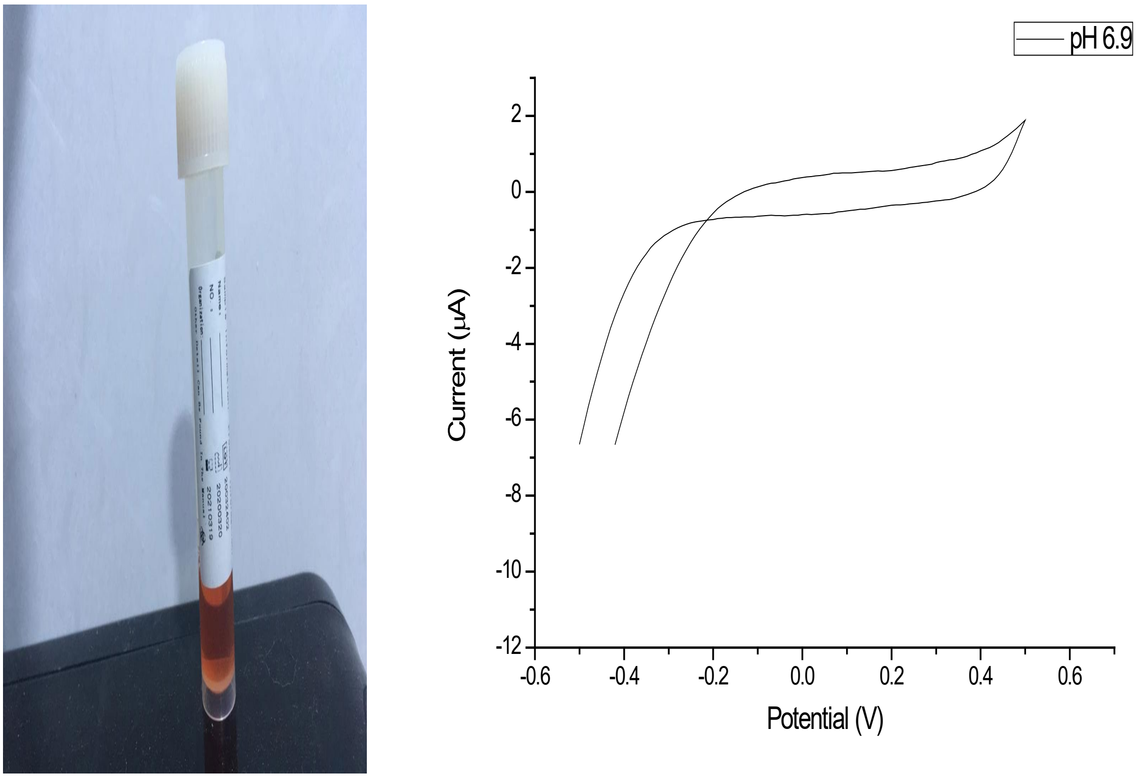

Figure 5: (A) Control VTM Sample, (B) Cyclic Voltammogram Recorded on Control VTM Sample; CV Behaviour of Control Reference Medium Which is Shown on the Graph

The values of relative changes of cathode current ΔIc/Ic=(Ic−Iref)/Iref at−0.5 V of all three level of pH was plotted in Figures. 6,7 and 8 against the value of each pH level. As one can see the behaviour of each pH value are completely different: ΔIc/Iref goes up with the increase in pH level for all values of VTM which appeared that ΔIc/Iref is almost flat at low level of pH and slightly increases at high value. This means that currents are practically not increased with low value of pH but increased at high pH value. This is a very promising result showing a possibility of pattern recognition of pH value using three levels of it.

4. Discussion

This study tested 18 specimens of VTM using electrochemical sensing assay. The effect of pH changes of all three different types of pH levels first lower than 7.0, 7.0 and more than 7.0 i.e. acidic, neutral and basic, on the screen printed gold electrodes, was studied with cyclic voltammetry. A typical series of CVs recorded on several VTM samples are shown in Figure 3. The CV curves in Figure 4A are almost featureless in the selected voltage range from -0.5 V to +0.5 V, which was chosen in order to avoid electrochemical reactions on the electrodes, with the scan range limited to where both cathodic and anodic currents just began to rise. The values of both cathodic and anodic current at -0.5 V and +0.5V, respectively, depend on the pH level changes in VTM solution, however the effect on anodic current is more pronounced and it was therefore used for analysis in this work.

CV cycles shift progressively upwards upon increasing the pH levels from 6.5 to 9 (Figure 3A-F). The characteristic parameter in this study, e.g. the value of anodic current at +0.5 V, increases with the increase in pH level for all samples. This means that the electrical conductivity is controlled by chemical reaction and the pH value on the surface of gold electrodes and acting as increasing the current. The correlation between pH value and the electric current (or conductivity) values is very important for further study of the effect of chemical mixture, and such measurements were always carried out separately [25]. The increase of pH value (in our case) causes the increase in both anodic and cathodic current [26], which is observed in Figure 4.

When analysing the effect of pH value on electrical properties of VTM solution, the values of anodic and cathodic currents (IA) at +0.5V from CV measurements were normalised by the currents values of bare electrodes in PBS with the addition of a particular value (IA0) to construct the values of relative changes of anodic and cathodic current. For example, each pH level was carried out separately and the reference was recorded on electrodes in VTM with 6.9 pH as control which produced in China. Figure.5

The relative changes in anodic and cathodic currents are presented in Figure 6. for all three pH values dependences of the pH changes level from low to high value. As one can see the effects of pH levels on screen printed gold electrodes are distinctly different. The electrode appeared to be affected by pH level of VTM even at low value since the $\Delta I_A/IA_0$ values increase monotonically in Figures (6 A,B) , (7 A,B) , and (8 A,B), respectively. This means that SPGE is equally promoted by increasing of pH value. then ΔIA/IA0 started to increase at different level of pH. Such behaviour of VTM as control was similar to those we studied.

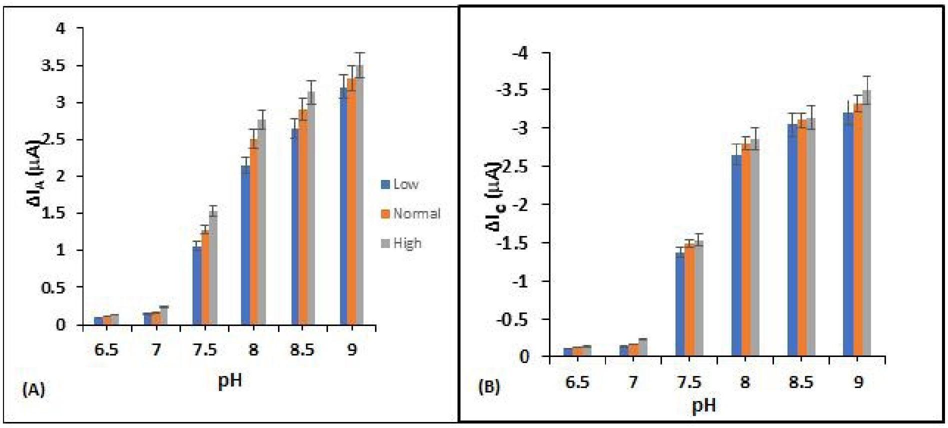

Figure 6: (A)The Dependence of Relative Changes of the Cathode Current at - 0.5 V for All VTM Samples with Different pH from 6.5-9.(B) The Dependence of Relative Changes of the Anode Current at + 0.5 V for all VTM Samples with Different pH from 6.5-9

(Figure 6 A,B) similarly to the other results of (7 A,B) , and (8 A,B) figures of pH studied, though the changes in $\Delta I_A/I_{A0}$ are more pronounced at high pH value, particularly for basic level. Moreover, an overall trend of small increase in $\Delta I_A/I_{A0}$ is observed. Such behaviour was expected since pH level can change due to the oxidation and reduction of the chemical reaction [27,28].

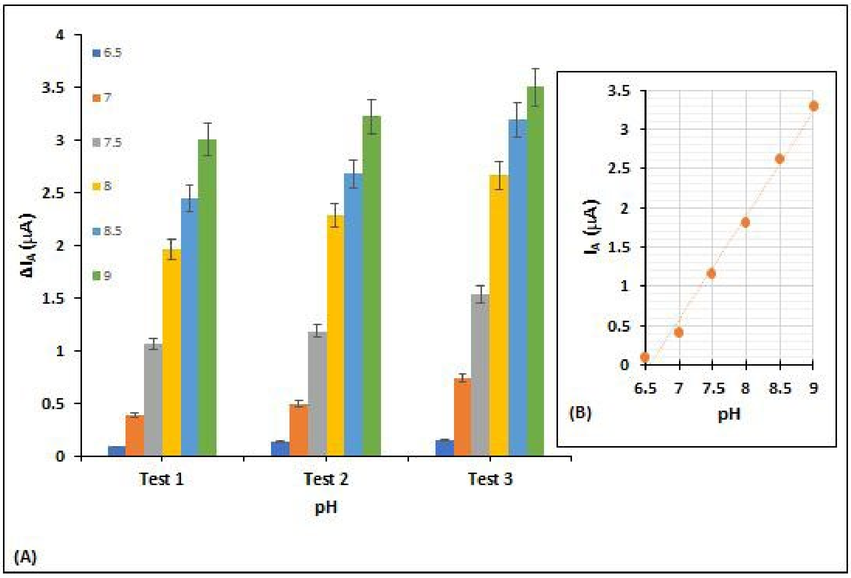

Figure 7: (A) Comparison of Relative Changes of Anodic Current (IA) at +0.5V of All Three Tests of VTM Samples on Surface of Screen Printed Gold Electrodes. (B) The Inset of Anodic Current (I_A) at +0.5V and pH Comparison Shows the Dramatically Increase

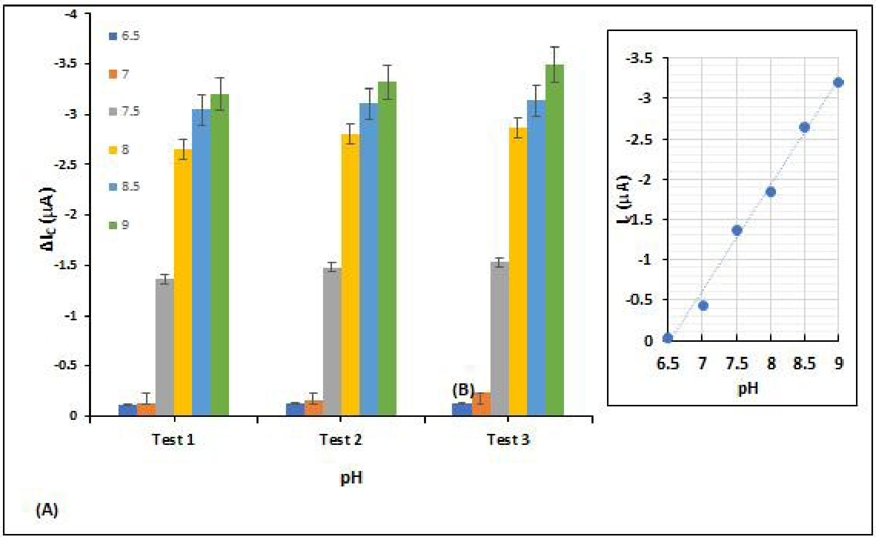

Figure 8: (A) Comparison of Relative Changes of Cathodic Current (Ic) at -0.5V of All Three Tests of VTM Samples on the Screen Printed Gold Electrodes Surface. (B) The Inset of Cathodic Current (I_c) at -0.5V and pH Comparison Shows the Dramatically Increase

The results presented in Figures 6, 7 and 8 show a possibility of pattern recognition the three types of pH value studied. The relative responses of the all three pH level to the VTM solution presented in a pseudo-3D plot in Figure 9, clearly demonstrated this outcome. The experimental points for acidic, neutral, and basic value shown in different colours are well-separated in the 3D-graph in Figure 9. This is a clear indication that pattern recognition principles can be applied for identification of each pH value using different types of pH level [29]. The value of VTM pH could be evaluated to using the appropriate calibration and data extrapolation [30].

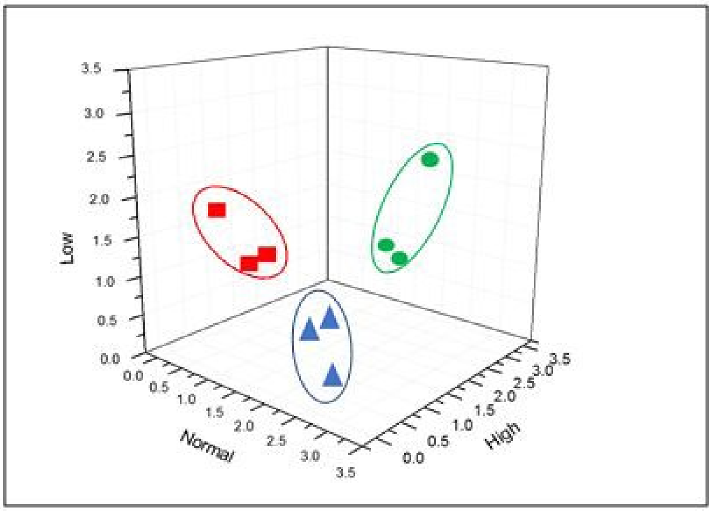

Figure 9: 3D Plot of Relative Changes in Anodic Current for Different VTM pH, Points with Different Colour Circles Show the Direction of the pH Value Increase from 6.5 to 7.5

5. Conclusions and Future Work

This study gives proof-of-principle for the use of a panel of diverse pH value to detect and evaluate different VTM sample. First of all, the values of anode (or cathode) current were found to correlate with pH changes and thus with the level of different VTM solution acting as promoter for conductivity. It shows simple electrochemical tests, i.e. cyclic voltammograms, on screen printed gold electrodes with VTM solution have distinctive characteristic responses to different pH level, and the pattern recognition principles can be applied for identification of VTM value.

This work paves the way for the development of novel, simple, and cost effective electrochemical based sensor array for preliminary assessment of the presence of virus in VTM solution. Future work which is currently underway will focus on using advanced data processing tools such as (ANN) Artificial Neuron Network for analysis of real virus transport medium samples with pathogen.

Author Contributions: H.F-M and H.F-H carried out majority of experimental work, also developed the concept and involved in discussion and writing and A.A. was involved in all aspects of work, but mostly in data analysis and writing. Both authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: There is no conflict of interests.

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