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Potentiometric Study and Statistical Analysis of Human Urine Samples using Reduced Graphene Oxide Screen Printed Electrodes

Anil B. Patil[1,*], Umesh. J. Tupe[2], Vikas V. Deshmane[3], Dharma K. Halwar[4], Sunil J. Patil[5] and Arun V. Patil[6,*]

[1]Department of Electronic Science and Research Centre, L.V.H College, Nashik, India
[2]Department of Electronics, Panchavati College of Management & Computer Science, Nashik, India
[3]Department of Physics, SICES Degree College, Ambarnath, Dist.-Thane, India
[4]Department of Electronic Science, MSG College, Malegaon, Dist.-Nashik, India
[5]Department of Physics, L.V.H College, Nashik, India
[6]Professor, Arts, Science and Commerce College, Manmad, Dist.-Nashik, India

ABSTRACT

In this work, the reduced Graphene Oxide (rGO) based screen printed electrodes (SPEs) were fabricated by using standard screen-printing technique to study electrical response of human urine samples. Under-test solutions of randomly collected human urine sample from different persons with different health condition were tested with the fabricated SPEs. For all tests, variation of output signal was noted for 1 minute at the interval of 2 seconds. For the urinalysis of collected samples, ion selective potentiometric method to sense generated electric signal was used which shows that electric potential of sensing layer change caused by adsorption due to hydrophobic or hydrophilic nature of urine samples of normal and diabetic persons. From the histogram and boxplot, we observed that the distribution seems to be normal for both normal person (range of -138.80 mv to -6.60 mV) as well as diabetic patients (range of 19.81 to 586.66 mV). So, we have performed two-sample t-test to check the significance within these two samples. We compare our test statistic i.e. p-value < $2.2e^{-16}$ with a critical value ($p < .05$). It is found that true difference in means is not equal to 0 and our results fall within the acceptable level of probability and hence, we conclude that there is difference between the two samples which will be useful for testing of human urine samples.

KEYWORDS: Urinalysis, rGO, SPEs, Potentiometric method, t-test.

INTRODUCTION

Urine is a sterilized liquid comprising of water, urea, and salts, which is by-product of the body. This is secreted by the kidneys through a process called urination. Urinalysis is a standard clinical analysis method used to study the physical or chemical components of urine which helps to understand the processes within the body for several disease conditions. Physical appearances like pH, density, colour, odour, and transparency of urine samples are prominent and distinguishable by vision only but, laboratory testing is required for few of them. Abnormalities in any one of these features will be the symptoms of minor problem in health condition of a person but can actually be indication of severe diseases, like diabetes. Diabetes could be a metabolic disease that affects the body's capability to either produce or use insulin. This is caused by the presence of high glucose levels resulting either weakened insulin excretion or malfunctioning insulin action, or both[1, 2]. Body utilize the glucose for energy in the form of insulin.

Approximately 422 million people worldwide are diabetic with a very high incidence every year as per report of World Health Organisation. Diabetes leads to blood glucose that upsurges to unusually high levels. Glucose level in healthy persons' urine is in the smallest amount typically 0 to 0.8 mmol/L. Presence of higher glucose level in urine sample is the indication of un-healthiness. Diabetes is that the most typical reason behind elevated glucose levels. It also merely reflects the state of your glucose over the prior few hours. If urine samples of persons shows pre-diabetic symptoms, more clinical tests of glucose level for diagnosis of diabetic condition of person will be required, but when neglected, led to severe disease related to diabetes, like neuropathy, nephropathy, retinopathy, and disorder, which arise in both type 1 and sort 2 diabetes, are core factors of severe morbidity, mortality, and big economic burdens[3–8]. Therefore, screening at an early stage is vital for the management of diabetes persons. Evaluation of glucose levels as screening and diagnostic criteria for diabetes[9]. However, both biomarkers have limitations. For blood sugar, fasting for a minimum of 8 h is required.

When quick treatment responses are essential, a urine glucose test which may be a non-enzymatic way for the testing and monitoring of diabetic. Additionally, people that potentially had diabetes could use the urine testing as the simplest method of measuring and monitoring the effectiveness of treatments to control glucose level.

Various techniques like Raman spectroscopy, chromatography, fluorescence and electrochemical-based aptasensors, immunoassay, and capillary electrophoresis etc are developed to look at the presence of glucose[10–14]. For screening and treatment of various diseases, detection and determination of biomolecules are clinically very important. Among these techniques, electrochemical platforms have attracted much attention due to their high sensitivity, rapid evaluation protocols, and value effectiveness.

From the literature survey, enzymatic biosensor[15-18] shows strong performances with accurateness and lower limit of detection. These are widely used for detecting biomolecules reinforced their electrochemical response with the enzyme, which is immobilized on the electrode surface. However, there are some drawbacks of enzyme-based electrodes, like their variability, the high price of enzymes and thus the issue of immobilization. Furthermore, toxic chemicals, temperature, and pH also affect the activity of enzymes. Therefore, non-enzymatic biosensors[19] gain the considerable attention for electrochemical detection of clinically important biomarkers fabricated using graphene-based nanomaterials due to high sensitivity, ease miniaturization, simple operation, speedy and low cost[20, 21]. Portable and disposable screen-printed electrode (SPE) in electrochemical sensors for study of urine sample offers the advantages over the majority electrode including avoiding the issue of cleaning electrodes, renewable surface, and thus the reduction of infection. Furthermore, analysis is usually done by single drop of sample onto electrode without the commonly used cumbersome electrodes and cells [22, 23]. Due to the electroactive nature of the many electrochemical sensors, Graphene based nanomaterial modified electrode are developed for the acid determination to beat the low selectivity of non-enzymatic electrochemical sensor[24].

Reduced Graphene Oxide (rGO) based biosensors have high electrical conductivity, light weight, high specific area, strong mechanical strength, and chemical stability, thermal, and biocompatibility properties, biomolecule adsorption through π-stacking interactions, induce strong substrate–molecule coupling, stable to biodegradation than enzymatic system, special molecular structure, and cheaper[25-27]. Because of the big extent, electrical conductivity, and high electron transfer rate of those molecules, additionally as their capacity to immobilize various molecules and facilitate chemical processing[28]. Hence, graphene is being increasingly applied as a sensor particularly in medical and biological applications[29, 30]. Hence, the event of an alternate method for the fast, disposable, and real-time indication of glucose level remains needed. Therefore, rGO SPEs are proposed to attain the sensitive, selective, portable and disposable non-enzymatic determination of glucose present within the urine samples of normal and diabetic persons.

The objective of this paper is to check the relevance of electrical conductivity, random samples of urine of various persons viz: male, female and kids; a unique promising rGO screen printed electrode with excellent potentiometric properties was prepared with high sensitivity, simplicity and speedy. This device will be useful for applications in the testing of human urine samples.

EXPERIMENTAL

Development of screen-printed electrodes (SPEs): Commercially available AR grade (99.99 % purity) graphene oxide (GO) powder was heated in inert atmosphere at 2000C for 1hr. After confirmed by EDS, thermally treated powder was used as rGO. On clean glass substrates of 5 cm X 1 cm, rGO working electrodes were developed by screen printing technique. Initially, all glass substrates were thoroughly washed with double distilled water and acetone then put under an IR lamp for 30 minutes to eliminate impurities. Silver ink was used to grow electrical contact of 1.2 cm X 1 cm on top of a glass substrate for external connection. For developing SPE, the sensing material was prepared using 70 % inorganic and 30% organic composition. Inorganic materials included rGO powder and organic materials included ethyl cellulose as a local binder to get the stickiness on substrate and butyl carbitol acetate (BCA) as vehicle for paste formation. In a mortar and pestle, the rGO powder and ethyl cellulose were combined and crushed for an hour, and then BCA was added drop by drop to create a thixotropic paste. This paste was uniformly spread using a screen-printing setup on lower portion of glass substrate with 4 cm X 1 cm area. Similarly screen printed reference electrodes were developed using silver ink on other glass substrates of 5 cm X 1 cm area. The developed SPEs were kept under IR irradiation for 45 minutes to eliminate local binder or impurity residue, and then these electrodes were used for further work.

Experimental setup: Experimental setup for urine sensing and sample data collection at a room temperature with the rGO based SPE is shown figure 1. Urine sample from different persons with different health condition were randomly collected and tested in the laboratory environment. The sensing area of the SPE was dipped care-fully into the test solutions to sense the conductivity. Care was taken that the contact terminals do not

touch the test solution. A digital voltmeter is used to measure the conductivity signal from the SPEs which is further fed to a computing unit for urinalysis of randomly collected samples. In present work, to evaluate the response of SPE to sense the urine, 10 different randomly collected samples out of 80 samples were studied.

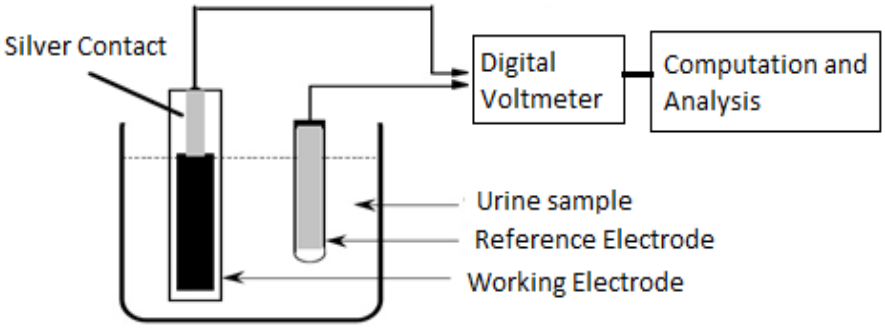

Fig. 1: Experimental Setup for Taste Sensing

Preparation of samples: Out of 80 samples of human urine of male, female and kids volunteer with normal and diabetic health condition, 10 samples were randomly selected and refrigerated immediately for testing purpose. The solution was transferred into the electrochemical cell for analysis without any further pre-treatment. The potentiometric voltage–time measurements and statistical analysis were carried out to study urine samples of the human.

RESULT AND DISCUSSION

Potentiometric Characterizations for Electrical Response: In this present work, the potentiometric electromotive force (EMF) signal recorded after dipping into the test solution of randomly collected urine sample from 10 different persons with different health condition to study the variation of output electric signal noted for 01 minute time duration at the interval of 02 sec.

Figure 2 shows plot of electrical output (mV) against the testing time for randomly collected urine samples. Electrolytes that exist in form of ions in the urine samples are glucose, uric acid, inorganic phosphorus, chlorine, calcium sodium and potassium. In rGO based working electrode of potentiometric sensor, interaction of different electrolyte molecules present in human urine samples changes the local charge carrier mobility.

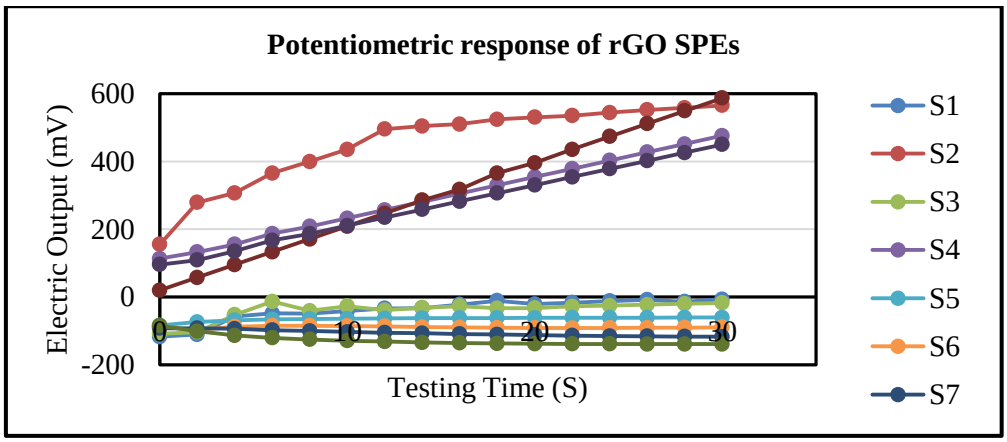

Fig. 2: Plot of electrical output (mV) against the testing time human urine samples.

This results in either increasing or decreasing the concentration of electrons reckoning on the character of electrolytes electron donor or acceptor species which further results in decrease or increase in electrical conductivity[31] as shown in above figure 2. In present electrically connected potentiometric circuit, positive ions in the electrical path travel to the cathode and negative ions travel to the anode to respectively produce oxidation reduction reactions. Different kidney functions decide the level of the conductivity of the urine which could be used as a parameter in routine urinalysis [32].

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Statistical Urinalysis: Figure 3 shows histogram of randomly collected human urine samples of (a) Normal and (b) Diabetic persons including male, female and kids. Out of ten human urine samples, six normal samples and four diabetic samples viz: S1, S3, S5, S6, S7 and S9 indicates urine samples collected from normal persons, while, S2, S4, S8 and S10 indicates urine samples from diabetic persons were used for urinalysis.

When the difference between two population averages is being investigated, a statistical t-test for the hypothesis testing is employed. Results are expressed as the mean values and standard deviation. The statistical evaluation was applied by analysis two-sample t-test. The test statistic that a t test produces may be a t-value [33-37].

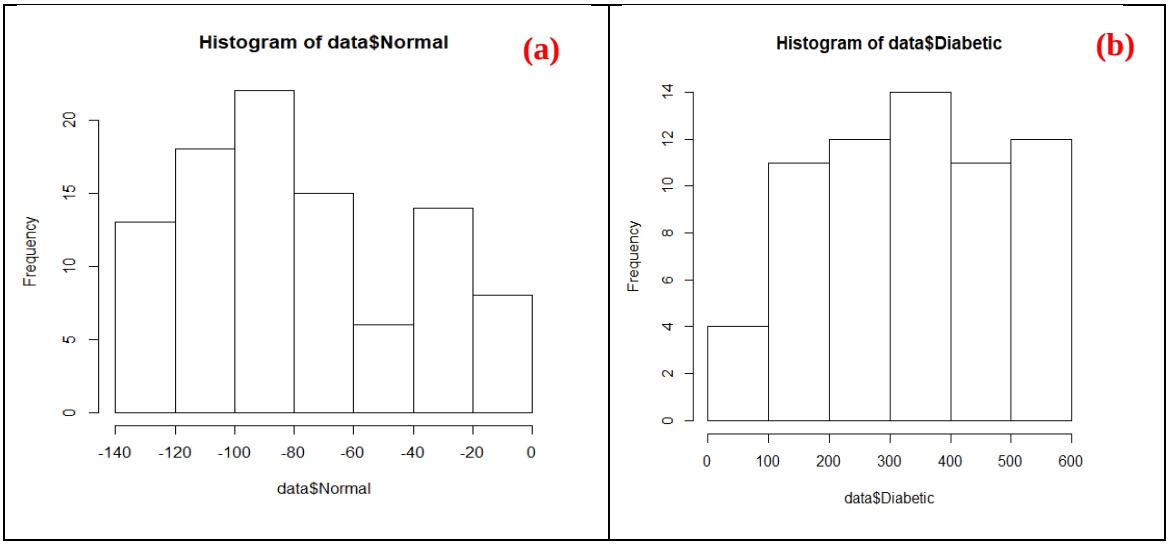

Fig. 3: Histogram of human urine samples of (a) Normal and (b) Diabetic persons.

In this present study, t = -21.142 and df = 68.546. When we assume a standard distribution exists, we will identify the probability of a specific outcome. We specify the extent of probability (level of significance, p). Values of $P < 0.05$ were considered as significant. We compare our test statistic i.e. p-value < $2.2e^{-16}$ with a critical value ($p < .05$). It is found that true difference in means isn't adequate to 0 and our results fall within the appropriate level of probability. 95 percent confidence interval between -445.8943 and -368.9920 found. Sample estimates mean of normal persons is -77.36224 and mean of diabetic person is 330.08094.

A boxplot may be a suitable method which can tell you about your outliers and what their values are, if your data is symmetrical, how tightly data is grouped and the way data is skewed. This plot shows the distribution of information supported a five-number summary ("minimum", first quartile (Q1), median, third quartile (Q3), and "maximum").

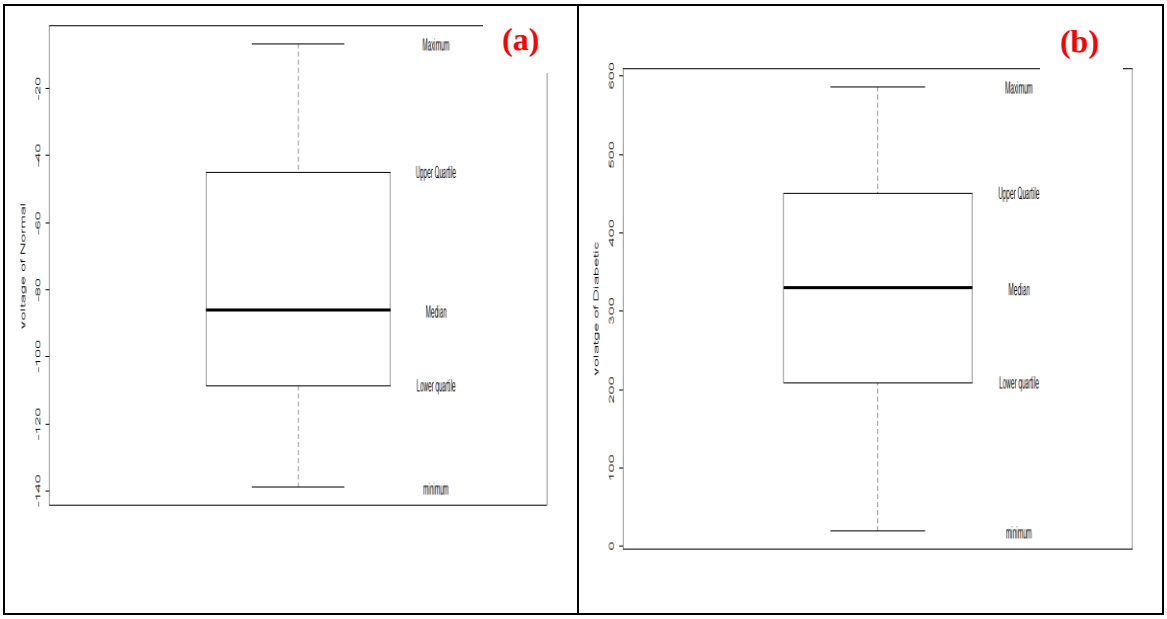

Fig. 4: Box plot of human urine samples of (a) Normal and (b) Diabetic persons.

In present study, the results showed that the human urine samples from normal persons were within the range of -138.80 mv to -6.60 mV, whereas those in patients with diabetes ranged from 19.81 to 586.66 mV (Table - 1), suggesting that the developed rGO SPEs has the potential to screen for diabetic persons.

Table 1: Summary of statistical data of Electric Responses to human urine samples

Urine Sample Source	Statistical data of Electric Responses to different urine samples						
	Mean	**Standard Deviation**	**Minimum**	**Maximum**	**Q1 (Lower Quartile)**	**Q2 (Median)**	**Q3 (Upper Quartile)**
Normal Person	-77.36223	12.34817	-138.80	-6.60	-108.20	-86.10	-46.67
Diabetics Person	450.17	31.68198	19.81	586.66	208.56	329.89	-46.67

CONCLUSION

The reduced Graphene Oxide based screen printed electrodes were successfully fabricated by using standard screen-printing technique for potentiometric study to investigate electrical response and statistical analysis of human urine samples. Under-test solutions of randomly collected human urine sample from male, female and kids with different health condition (Normal and Diabetic persons) were tested with the fabricated SPEs. For all tests, variation of output signal was noted for 1 minute at the interval of 2 seconds. Ion selective potentiometric method shows that electric potential of sensing layer change caused according to nature of urine samples of normal and diabetic persons.

From the histogram and boxplot, the results showed that the human urine samples from normal persons were within the range of -138.80 mv to -6.60 mV, whereas those in patients with diabetes ranged from 19.81 to 586.66 mV, therefore, we observed that the distribution seems to be normal for both normal as well as diabetic patients. So, we have performed two-sample t-test to check the significance within these two samples. We compare our test statistic i.e. p-value < 2.2e-16 with a critical value ($p < .05$). It is found that true difference in means is not equal to 0 and our results fall within the acceptable level of probability and hence, we conclude that there is difference between the two samples. From t-test, p-value is almost zero which is less than 0.05 (level of significance) and hence we conclude that there is difference between the two samples. The findings revealed that results of human urine samples obtained by the proposed rGO based SPEs were consider as promising tool in urinalysis applications.

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REFERENCES

1. N. H. Cho, J. E. Shaw, S. Karuranga, Y. Huang, J. D. da Rocha Fernandes, A. W. Ohlrogge and B. Malanda, *Diabetes Res. Clin. Pract*, **138**, 271-281, (2018).
2. L. Jaspers, V. Colpani, L. Chaker, S. J. van der Lee, T. Muka, D. Imo, S. Mendis, R. Chowdhury, W. M. Bramer and A. Falla, *Eur. J. Epidemiol*., **30,** 163-188, (2014).
3. K. H. Wagner and H. Brath, Prev. *Med.,* **54,** S38-S41, (*2012).*
4. S .Van Dieren, J. W. J. Beulens, Y. T. Van Der Schouw, D. E. Grobbee and B. Neal, Eur. *J. Cardiovasc. Prev. Rehabil*., **17,** S3 - S8, (2010).
5. M. N. Piero, Asian J. Biomed. Pharm. Sci., **4,** 1-7, (2015).
6. B. W. Stewart and C. P. Wild, *World Cancer Rep*., 1- 8, (2014).
7. A. Boutayeb and S.Boutayeb, *Int. J. Equity Health*, **4,** 1- 8, (2005).
8. T. Gan, X. Liu and G. Xu, *Kidney Int. Rep*., **3**, 542 – 554, (2018).

9. N Alqahtani, WAG Khan, M. H. Alhumaidi and, YAAR Ahmed. *Int. J. Prev. Med.*, **4**, 1025 – 1029, (2013).
10. K. Ikeda, Y. Sakamoto, Y. Kawasaki, T. Miyake, K. Tanaka, T. Urata, Y. Katayama, S. Ueda and S. Horiuchi, *Clin. Chem*., **44**:256 – 263, (1998).
11. K. Inoue, A. Goto, M. Kishimoto, T. Tsujimoto, R. Yamamoto-Honda, H. Noto, H. Kajio, Y. Terauchi and M. Noda, *Clin. Exp. Nephrol*., **19**, 1179 – 1183, (2015).
12. N. C. Dingari, G. L. Horowitz, J.W. Kang, R .R. Dasari and I. Barman, *PLoS ONE*, **7,** e32406, (2012).
13. D. J.S. Hinton and J. M. Ames, *Int. Congr. Ser*., **1245,** 471- 474, (2002).
14. C. Apiwat, P. Luksirikul, P. Kankla, P. Pongprayoon, K. Treerattrakoon, K. Paiboonsukwong, S. Fucharoen, T. Dharakul and D. Japrung. *Biosens. Bioelectron*, **82,** 140 – 145, (2016).
15. T. Madasamy, C. Santschi and O. J. Martin. Analyst, **140,** 6071 – 6078, (2015).
16. T. Madasamy, M. Pandiaraj, M. Balamurugan, S. Karnewar, A.R. Benjamin, K. A. Venkatesh, K. Vairamani, S. Kotamraju and C. Karunakaran, *Talanta,* **100,** 168 – 174, (2012).
17. T. Madasamy, M. Pandiaraj, M. Balamurugan, K. Bhargava, N.K. Sethy and C. Karunakaran, *Biosens. Bioelectron*, **52**, 209 – 215, (2014).
18. M. Pandiaraj, T. Madasamy, P. N. Gollavilli, M. Balamurugan, S. Kotamraju, V.K. Rao, K. Bhargava and C. Karunakaran, Bioelectrochemistry, **91,** 1 – 7, (2013).
19. L. Li, Z. Du, S. Liu, Q. Hao, Y. Wang, Q. Li and T. Wan,. Talanta, **82**, 637–1641, (2010).
20. B. Fang, Y. Feng, G. Wang, C. Zhang, A. Gu and M. Liu, *Microchimica Acta*, **173(1-2),** 27 – 32, (*2011)*.
21. C. L. Sun, C.T. Chang and H. H. Lee, *ACS Nano*, **5(10),** 7788 – 7795, (2011).
22. L. Fritea, M. Tertis, C. Cristea, S. Cosnier and R. Sandulescu, *Analytical Letters*, **48(1),** 89 – 99, (2015).
23. A. R. Rajamani, R. Kannan and S. Krishnan, *Journal of Nanoscience and Nanotechnology*, **15(7),** 5042 – 5047, (2015).
24. J. Du, R. Yue and Z. Yao, *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, **419,** 94 – 99, (2013).
25. L. Fu, G. Lai, G. Chen, C. Lin and A. Yu, *Chemistry- Select*, **1(8),** 1799 – 1803, (2016).
26. W. B. Choi and J. W. Lee, *Taylor & Francis Group, LLC,* (2012).
27. C. R. Sekhar, *Elsevier Inc.* (2015).
28. C.N.R. Rao, A.K. Sood, K.S. Subrahmanyam and A. Govindaraj, *Angew Chemie Int. Ed*., **48,** 7752 – 7777, (2009).
29. A. H. Loo, A. Bonanni and M. Pumera, *Analyst,* **138,** 467 – 471, (*2013)*.
30. A. H. Loo, A. Bonanni and M. Pumera, *Nanoscale,* **5**, 7844 – 7848, (*2013)*.
31. T. Ashutosh and S. Mikael, *Scrivener Publishing*, (2015).
32. Pet Healthcare, Senno Technology Inc, Zhonghua Rd. Hsin Chu City, 300, Taiwan, No. 516, Sec.4.
33. A. A. Megahed and P.D. Grünberg Walter Constable, J Vet Intern Med. **33,** 1530–1539, (2019).
34. R. Deanne, Thesis for Master of Science, College of Nursing, The University of Utah, (1980).
35. H. G. Oh, D. C. Jeon, M. S. Gianti, H. S. Cho, D. A. Jo, M. N. Indriatmoko, B. K. Jang, J. M. Lim, S. Cho, and K. S. Song, Nanomaterials, **11,** 787, (2021).
36. S. Thara, S. Chutintorn, K. Taya and K. Teerakiat. The 2016 Biomedical Engineering International Conference (BMEiCON-2016), IEEE, **978**-1-4673-9158-0/15, (2016).
37. W. Wassa, P. Thitirat, P. Dechnarong, C. Wireeya, S. Nuankanya, J. Deanpen, Biosensors, **11,** 85, (2021).