Armenian Journal of Physics, 2022, vol. 15, issue 4, pp. 131-140

Saliva Sensors

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(Received: September 29, 2022; Revised: October 15, 2022; Accepted: October 19, 2022)

Abstract. Saliva, an alternative body fluid that is easily accessible and contains trace amount of glucose can be potentially used for the noninvasive monitoring of diabetes. A positive correlation between blood glucose (BG) and stimulated salivary glucose (SG) in diabetics has been reported. The main goal of Self-Monitoring of Blood Glucose is to monitor a person's blood glucose at different time intervals which can aid a doctor in adjusting medication/insulin dose and is also useful to a patient in evaluating his/her response to therapy. Versions of saliva glucose (SG) sensor fabrication are reported. SG analysis with reference method was discussed. Salivary protein biomarkers are reported. Special strips or chips and saliva tests are presented.

DOI: 10.54503/18291171-2022.15.4-131

1. Introduction

Finger pricking as the main routine in these invasive techniques is troublesome for diabetic patients because it can lead to scarring, motivating the development of devices that enable glucose measurement to be done cheaply and in noninvasive way.

Glucose measurements are frequently necessary for the effective glycemic control and appropriate therapeutic interventions; therefore, an innovative, non-disruptive method using saliva as an indicative fluid is being explored to manage diabetes. Noninvasive blood glucose monitoring methods are based on measuring glucose concentration from its chemical, electrical, or optical sensing properties. Noninvasive medical procedures do not require the introduction of instruments into the body. Among corresponding techniques which are promising for analysis of different processes in living organism are absorption spectroscopy, Raman spectroscopy, fluorescence, surface plasmon resonance interferometry, optical coherence tomography, photoacoustic spectroscopy, transdermal techniques, impedance spectroscopy, reverse iontophoresis, electrochemical techniques, enzymatic detection of glucose, refractive changes in the eye, ultrasonic, electromagnetic and heat capacity etc. Some other sensing properties can also be exploited for measurement since the human body shows different physiological responses to changes in glucose, such as thermal electric and acoustic impedance, thermal conductivity, and electromagnetic response.

Usual classification of noninvasive methods is based on the subject they analyze, such as differentiation of media they target, including tissues (skin, aqueous eye humor, oral mucosa, tongue, and tympanic membrane) and fluids (sweat, urine, saliva, and tears). Several wearable and noninvasive methods are discussed based on monitoring the interstitial fluid and wearable devices based on detection of the sweat (eyeglass, flexible wristband, etc.), breath analysis, saliva analysis (tattoo printed on a tooth, etc.), and ocular fluid (smart contact lens).

Saliva has a great role in the homeostasis of the oral cavity because it stabilizes the ecosystem of the oral cavity; and hence, it serves as a marker to timely discover the disease that further leads to more successful treatment, risk estimation, estimation of glucose level and it is a simple, noninvasive alternative to blood and urine tests [1, 2]. Saliva is said to be the ultra-filtrate of blood. Glucose is one of the blood components that are transferable across the salivary gland epithelium in proportion to its concentration in blood. Saliva generated from each gland has unique rheological

properties with different diagnostic capabilities that can be extracted. For instance, the parotid gland, which has lower viscosity (1-3 mPa), is usually separated and taken for clinical analysis. A positive correlation between blood glucose (BG) and stimulated salivary glucose (SG) in diabetics has been reported. [3]. Saliva, an alternative body fluid that is easily accessible and contains trace amount of glucose can be potentially used for the noninvasive monitoring of diabetes.

In fact, some commercially available saliva-based devices include testing for drugs and alcohol, antibodies, the human immunodeficiency virus, and steroids [1, 2]. Saliva uses as a diagnostic fluid. Whole saliva offers distinctive advantages over blood: it is easily accessible and good for rapid tests; it contains multiple biomarkers enabling multiplex detection; the noninvasive tests can be brought to point-of-care locations, such as for home use and especially for children and elders. A positive correlation between SG levels and BG/serum glucose levels is established. The variability in SG levels of different workers may also be a reflection of different choice of study design, as well as the diversity of the methods and criteria for selecting the samples. Studies reveal diabetics are distinguished from healthy subjects based on their higher average SG levels of about 8.2 mg/dL for diabetics as compared to 1.7 mg/dL for healthy persons [4-6].

Curiously, it is also proposed to use conventional glucose meters to diagnose coronavirus through saliva. Such a test has 100% accuracy, moreover, it can be used at home to determine the coronavirus - with the help of a glucose meter, the patient will be taken saliva with a cotton swab, as during a normal test.

2. Glucose monitoring

According to the World Health Organization, currently there are around 450 million peoples suffering from diabetes in the world, and this number could potentially reach 700 million by 2045. The number of patients with diabetes mellitus in Armenia today is 73 thousand [7].

Therefore, monitoring of glucose levels on a daily basis is important, especially for individuals with confirmed diabetes, in order to avoid various complications associated with the disease.

The main goal of Self-Monitoring of Blood Glucose (SMBG) is to monitor a person's blood glucose at different time intervals which can aid a doctor in adjusting medication/insulin dose and is also useful to a patient in evaluating his/her response to therapy [8]. The most common method of SMBG involves finger, but, in recent years, saliva, tears, urine etc. have also emerged as alternatives to blood [9].

Various groups around the world have been working on non-invasive diagnostics in the field of glucose monitoring. Agrawal et al. tried to correlate blood glucose and saliva glucose concentration using known and commercial methods [3]. Claussen et al. fabricated an electrochemical biosensor by to detect glucose in saliva and tears using nanosheets of graphene, platinum nanoparticles and glucose oxidase enzyme (GOE) [10]. Similarly, blood glucose levels measured using midinfrared quantum cascade laser spectroscopy [8]. Wang et al. described a saliva glucose monitoring system using carbon nanotubes functionalized with metal nanoparticles, polymer layers and GOE [9]. However, major drawbacks of these sensors are involvement of complex procedures. Most of these developed sensors are expensive and bulky in nature, hence can't be used for onsite diagnosis conveniently, except for the commercially developed iQuickit Saliva Analyzer.

It was consistently felt across the research community that among these non-invasive body fluids, saliva was most significant in glucose determination because of its good correlation with blood glucose as reported by various groups [11-13]. A paper strip based glucose biosensor had developed for non-invasive determination of salivary glucose by scanning color changes.

Recently, smartphones have emerged as portable, inexpensive and convenient platforms for point of care testing of various analytes. More than 40,000 mobile health applications are presently

available and their number is increasing rapidly. Most of these small, self-contained, standalone devices contain a high quality built in camera and possesses computational features; most of these are GPS enabled which integrate them to various public health projects.

Thus smartphones act as robust, inexpensive, miniaturized systems which can help achieve the goal of "personalized medicine" where a layman can easily conduct the testing himself at home with limited training and also lead to in situ diagnosis in poor as well as remote areas devoid of conventional equipment and health facilities [14].

Such diagnostic methods include that for lactate [15, 16], cortisol [17] and biomarkers for diseases such as cancer [18-21] in non-invasive body fluids as well as serum. Both optical as well as electrochemical techniques have been employed for detection. An optical smartphone based biosensor for glucose has been developed by Chun et al. where they have fabricated the strips using wax printing and microfluidics and color changes have been detected using Image J software. Testing of the biosensor has been carried out in human serum [20]. Some smartphone based glucose monitors have also been commercialized existing smartphone based apps also had limitations such as lack of personalized feedback, security issues and usability issues such as data entry and integration with patients and electronic health records [22].

Therefore, after weighing in the drawbacks of reported noninvasive sensors and intended usability, portability and accuracy of the device, a standalone smartphone based optical biosensor for the detection of glucose in saliva samples was developed.

3. Versions of saliva glucose (SG) sensor fabrication

Versions of saliva glucose (SG) sensor fabrication reported in Ref. 34-37 in [23]. Let us to report shortly these versions below. DS550 screen-printed electrodes (Dropsens incorporated, Asturias, Spain) were used as sensor chips. In this study, researchers developed a simple and disposable saliva-based biosensor for real-time SG monitoring. It contains a screen-printed sensor chip and the working electrode is functionalized with single wall carbon nanotube (SWCNT) and 3 layers of (CS/GNp/GOx) multilayer films. SG sensors displayed a linear detection range of 0.5-20 mg/dL glucose in Public Pharmaceutical Scheme (PBS) with a detection limit of 0.41 mg/dL. A layer-by-layer self-assembly modification process was applied to functionalize the working electrode surface. Multilayer films were composed of SWCNTs functionalized with carboxylic groups (SWNT-COOH, diameter: 1~2 nm; length: 2~5 μm, Brewer Science, Rolla, CA) and three repeated layers of chitosan (CS)/gold nanoparticles (GNp)/glucose oxidase (GOx) to achieve the best glucose sensing performance. 2 mg/dL CS were prepared by dissolving the CS powder thoroughly into acetate buffer solution (pH 4.65). GNp (20nm diameter) was used as received. GOx (type II lyophilized powder with at least 17,300 units/g solid) was dissolved in phosphate buffered saline (PBS, pH 7.4) to produce 1 mg/mL enzyme stock solution. Also, the sensors detected 1.1-45 mg/dL glucose in saliva samples. A study of 10 healthy subjects accomplished a SG detection range of 1.1-10.1 mg/dL with acceptable accuracy against standard reference method as evaluated. 89% of sensor tests demonstrated less than 20% difference against reference values, and the remaining 11% of sensor tests were considered acceptable for their intended uses. Results indicated (1) the individual BG/SG ratio at fasting was relatively consistent over time if subjects' health conditions were unchanged; (2) the individual SG levels tracked closely with BG levels after meals; (3) a time difference of 15-30 minutes between peak levels of BG and SG was observed due to physiology of the body metabolism; (4) the individual BG/SG ratio 2 hours after meals returned to a similar value at fasting. These discoveries showed great potential for diabetics to manage glycemic levels by simply measuring their SG levels at fasting, before and 2 hours after each meal, and before sleep. The saliva test has great potential to be applied as an adjunct diagnostic method for glucose monitoring in the future. Eventually, with a full

clinical validation involving patients with diabetes, medical health providers and patients could use the SG sensors for diabetes screening.

According to results from healthy human subject research, the lowest SG level obtained was 1.1 mg/dL, while the highest was 10.1 mg/dL. However, since patients with diabetes' SG level can reach down to 0.3 mg/dL during hypoglycemia condition, and raise higher than 20 mg/dL during hyperglycemia condition, a broader SG measurement range is in need to be verified to warn patients from these dangerous situations. Also, a shelf life of at least 6 months is required to apply sensors in clinical exercise. In the future, rugged packaging needs to be designed to protect sensors and retain bioreactivity during storage, so as to achieve longer shelf life for such biosensors.

After fabrication, all sensors were packed in gel-boxes (Gel- Pak, Hayward, CA) and sealed in vacuum bags using a vacuum packaging machine (VACmaster pro110, Overland Park, KS). Sensors were preserved at 4°C when not in use.

Saliva glucose (SG) sensing has long been considered a noninvasive alternative to blood glucose monitoring for diabetes management. The sensor utilizes glucose dehydrogenase flavine-adenine dinucleotide (GDH-FAD) in conjunction with disposable screen printed electrodes to measure glucose levels in saliva. A disposable SG sensor without any requirement for sample preparation was used. The lower limit of detection was determined to be 0.11 mg/dL. A lag time of 15 minutes with a positive correlation between SG and BG levels was found, which agrees with literature results (Fig. 4 [24]). The detected SG ranges from 2.38 to 3.40 mg/dL over a BG range of 90 to 143 mg/dL.

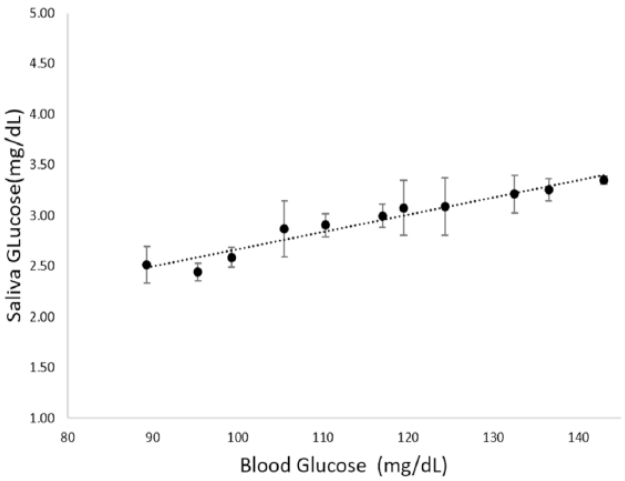

Fig. 1. A correlation between SG level and BG level.

This study reveals that salivary glucose is increased in diabetics. Significant of controlled diabetes, uncontrolled diabetes and healthy individuals. This means that saliva can be used as adjuvant diagnostic tool to blood in early diagnosis for diabet. Saliva also finds great advantage over blood in case of children, elderly, critically ill and debilitated patients. The standardized procedure of salivary glucose estimation for diabet may herald a new era in noninvasive method.

4. SG Analysis with reference method

A spectrophotometric method for quantitative detection of glucose was conducted to validate sensor measured SG levels. Ultraviolet-visible (UV-vis) spectroscopy (mini1240, Shimadzu, Kyoto, Japan) was used in [23] along with colorimetric glucose assay kits (K606-100, Biovision, Milpitas, CA) based on enzymatic reactions.

Saliva samples were boiled at 100°C in a BSH200 mini dry bath (Benchmark scientific, Edison, NJ) for 30 to 60 minutes to kill reactive biomolecules residua. After cooling down, cell

debris was removed by centrifuging at 12,000 × g for 6 minutes using an Eppendorf 5424 micro-centrifuge (Hauppauge, NY). Clear supernatant was collected and analyzed immediately by UV method. Otherwise, samples were stored at –20°C (no longer than 24 h) and thawed at room temperature before analysis. Pooled saliva was gathered from 2 healthy subjects and treated using the same procedure as for saliva samples. Six standard glucose solutions plus 1 blank sample (without reaction reagent) were prepared along with saliva samples to provide a calibration curve. Standard glucose solutions were prepared by spiking pooled saliva with different glucose concentrations to eliminate a matrix effect. Samples were then mixed with reagents from glucose assay kits following the manufacturer's protocol and incubated at 37°C for 40 minutes.

Fig. 2.

Absorbance measured at 570 nm wavelength was proportional to glucose levels. SG levels were then calculated using the calibration curve from this batch test.

5. Calibration of the biosensor and the estimation of glucose levels in saliva samples

Calibration of the biosensor was carried out in [25] using spiked saliva samples. Glucose concentration already present in saliva of the healthy donor was determined using spectroscopic method. Usually 10 s is the minimum time required for sample handling (sample application and absorption on the strip), thereafter response slope was obtained for 10 s (after initial 10 s of sample handling), thus giving a response time of 20 s total. Change in slope for red (R), green (G) and blue (B) pixels were obtained for different glucose concentrations and calibration curves were then plotted. As R, G and B together form an additive model of color change, different combinations of these colors were used and calibration curves were obtained against glucose concentration v/s change in slope within 10 s for all these combinations separately to increase the sensitivity. Interference studies were carried out using ascorbic acid and lactic acid which are commonly found interferents present in human saliva. For estimation of glucose in real sample, saliva sample was applied on the strip using a clean ear-bud and color changes were scanned on the other side of the strip. For estimating color changes in the strip through the app, the strip was placed inside a dark box after adding saliva sample; time for obtaining slope (10 s) was already fixed before adding saliva. Then the smartphone was placed over the box while the camera and flash light areas could oversee the strip through the hole. The app then automatically sensed the detection zone and calculated change in response slopes and displayed unknown glucose concentration according to the calibration curve equation fed into the app. Validation of the biosensor was carried out on both healthy and diabetic subjects.

In the present study, a smartphone based non-invasive optical biosensor has been developed for the estimation of glucose levels in saliva samples. The sensor was fabricated using a simple

methodology by immobilization of Glucose oxidase enzyme along with a pH indicator bromocresol purple on a filter paper based strip. The color change with respect to glucose concentration was determined using RGB profiling with our developed smartphone application based on slope method. Slope method has been chosen against differential method of obtaining response to increase the sensitivity and reduce ambient light interferences and also for the reason that no baseline correction was needed. Different modes of obtaining response were tested, such as effect of different dyes and smartphone models so as to get maximum response and increase accuracy of results. Calibration curve with (R þ G þ B) Slope was found to be most sensitive with sensitivity of 0.0012 pixels sec^{1}/ mg dL^{1} within the linear detection range of 50e540 mg/dL and LOD of 24.6 mg/dL within a response time of 20 s. Clinical validation of the biosensor has been carried out on both diabetic and healthy subjects where correlation was established between their salivary glucose (SGL) obtained through our biosensor and blood glucose (BGL) obtained using commercial Accu Chek active blood glucometer. A very good correlation of 0.94 has been established in case of diabetic patients while in healthy subjects it was not so significant. For better standardization of the biosensor, subjects were grouped into several categories based on age, sex, status etc. and correlation between BGL and SGL in all these cases has been carried out. The sensor was found to be free of interferents such as ascorbic, lactic acid and lactose within the clinically relevant range of these acids reported in human mouth. Other advantage of the developed sensor is that the procedure involved is simple where a person just has to obtain saliva in an ear bud and apply it on the strip; the procedure is painless and can be performed even by a layman with limited training. Moreover, results are obtained in less time (just 20 s) and screening is done through a smartphone which has become a common gadget nowadays in almost everybody's homes, thus eliminating the need for procuring any specialized instrument for analysis. The sensor also satisfies the ISO standards in terms of accuracy as the readings obtained were in zones A and B of Clarke's error grid. The overall analytical performance and sensor test strip cost compared well or better than those reported in literature.

6. Salivary Protein Biomarkers

Note that Saliva and its components, such as proteins and peptides, can be used as indicators of general health to measure risks of developing certain diseases including cancer and perhaps diabetes [26-30]. Unlike blood, except in the case of severe diabetic patients, there is virtually little or no glucose in saliva because salivary glandular cells re-absorb glucose to prevent overgrowth of micro-organisms in the oral cavity. Therefore, directly measuring salivary glucose is not a viable option. Salivary protein composition is, however, influenced by systemic changes in the plasma, especially glucose level. Systemic changes, including diabetes, can therefore be seen in the alterations of salivary protein expression profiles, or proteomes [29, 31].

Studies of salivary proteomes give rise to novel salivary protein biomarkers for diagnosing both oral and systemic diseases including cancer, infection, and cardiovascular disease [25-28]. Using mass spectrometry (MS)-based proteomic approaches, it was shown that when compared to controls, there are specific salivary biomarkers associated with type 1 and 2 diabetes [27, 32, 33].

Proteins in saliva may provide a reflection of plasma glucose status in relation to diabetic conditions, for instance the Hemoglobin A1C A1C/SMBG status. Selfmonitoring of blood glucose (SMBG) is a home monitoring method that provides only a single point measure of plasma glucose. Note that proteins are involved in biological processes relevant to diabetic pathology such as endothelial damage and inflammation. Moreover, a putative preventive therapeutic approach was identified based on bioinformatic analysis of the deregulated salivary proteins. Thus, thorough characterization of saliva proteins in diabetic pediatric patients established a connection between molecular changes and disease pathology. This proteomic and bioinformatic approach highlights

the potential of salivary diagnostics in diabetes pathology and opens the way for preventive treatment of the disease.

7. Special strips or chips

As a solution for real-time glucose measurements using saliva for diabetic care, some special strip or chip is necessary. Therefore, some complicate solutions with saliva should be prepared. For example, the sensor utilizes glucose dehydrogenase flavine-adenine dinucleotide (GDH-FAD) in conjunction with disposable screen printed electrodes to measure glucose levels in saliva collected should be prepared. Details of preparation of test strips and immobilization of glucose oxidase as well as development of smartphone application reported in [24]. Cyclic voltammetry and amperometric-time (Amp-it) assays were also used to develop calibration curves and test subjects.

Some strips consist of some lysis enzymes that can react with saliva. The strip contains enzymes that can kill all organic substances in the saliva other than glucose. For example, proteolytic enzymes cause lysis of proteins and lipolytic enzymes causes the lysis of lipids. The remaining glucose concentration in saliva should be calculated. The reaction between enzymes and saliva form hydrogen peroxide (H_2O_2). The measurement of hydrogen peroxide relays the value of salivary glucose intensity. The device requires an electrode that can measure the hydrogen peroxide value by passing current. IR spectroscopy is also used to detect glucose range. The amount of hydrogen peroxide can be measure using a biosensor unit. This biosensor acts as a transducer here. The hydrogen peroxide quantity is measured because it is not the direct measure of glucose amount in the saliva. The pre-amplifier may be used to amplify the transducer current output. Analog to Digital Converter (ADC) is used to get output in digital form. Note that the transformation of saliva into hydrogen peroxide takes place in the strip reported above.

An on-chip disposable glucose nano-biosensor have developed through a layer-by-layer assembly process. It was mentioned above, that multilayer films were composed of SWCNTs functionalized with carboxylic groups and three repeated layers of chitosan/gold nanoparticles/glucose oxidase to achieve the best glucose sensing performance. Nano-biosensor includes the micro-fabrication of the sensor chip and Berkeley Lab' assembly of sensors and electrode modification. The on-chip electrochemical sensing device contains at least one working electrode, a counter electrode and a reference electrode and one possible electrode. Electrochemical measurements and characterization Cyclic Voltammetry (CV) and amperometric measurements were also performed. Amperometric measurements for determining the salivary glucose levels were performed of filtered unstimulated saliva samples using a mini potentiostat. A Supra 25 Scanning Electron Microscope (SEM) was employed for the surface morphological characterization of the SWCNT, GNp, and GOx on the sensor electrode reactive region. UV Spectrophotometer measurements and viscosity measurements using uVISC™ Portable Viscometer Control Advanced System are necessary measurements for determining the salivary glucose levels.

Of course, saliva is a noninvasive, easily collected biological fluid. As can see from examples above mentioned simple individual non-invasive daily diabetes management by the patient himself outside the hospital or clinic is possible only by use of special strips. The glucometer using saliva planned to production in next years by Ines Zimmer company in Hamburg, Germany. It is painless, faster, simpler than other ones before, but it will have too high prize for mass use.

It was shown in [34] that although these new methodologies using Machine Learning and Neural Network Methods and Correlation with Heart Rate Variability may have promising results in terms of patients' comfort, they still lack the needed accuracy. In order to get a better understanding of the gathered measurement data, many of those measurement methods use Machine Learning and Neural Network techniques to achieve better accuracy. Expenses and proving benefit are probably those that need to be made more affordable and demonstrated in further research. However, it takes a lot of time to market the technology from one side and to

change the behavior of both the patients and doctors. The noninvasive methods are usually based on many methods, including spectrometry or analysis of other parameters correlated with the glucose level. Authors aim to present in paper the available methods and ongoing projects for noninvasive glucose measurement, focusing on the use of machine learning (ML) and neural network (NN) methods used in a lot of ongoing research to deal with estimation methods of the glucose level.

8. Saliva Tests

Non-Invasive, Saliva-Based Glucose Test for Diabetes Management proposed by iQ Group Global on February 4, 2019 is shown that the glucose measurement recorded by the biosensor will be presented via the proprietary digital app on the patient's smart device in real-time where the patients may also compare historic glucose levels. This will open significant opportunities to improve the way diabetes is monitored and managed, enabling patients to store and analyze their data, share monitoring data with their healthcare team or relatives, create and send automated reminders when it is time to test glucose levels, offer educational services, and act as a provider for healthcare companies who offer patient support programs. Self-management of blood glucose (BG) is considered as a solution for real-time glucose measurements using saliva for diabetic care. Self-management of blood glucose (BG) is considered. As a solution for real-time glucose measurements using saliva for diabetic care, on-chip disposable glucose nano-biosensor through a layer-by-layer assembly process has developed. Saliva, an alternative body fluid that is easily accessible and contains trace amount of glucose can be potentially used for the noninvasive monitoring of diabetes [23].

Note that glucose measurement is mostly classified by the level of invasiveness of the sensing devices, which are usually classified as invasive (devices that are implanted in the patient's body or that invade the body to access a blood sample), minimally invasive (devices that painlessly invade a very small part of the patient's body, such as skin to collect a minimal sample, like a skin part, sweat, tear, and saliva), and noninvasive devices (devices that do not invade the patient's body).

Pooled saliva was gathered from 2 healthy subjects and treated using the same procedure as for saliva samples. Six standard glucose solutions plus 1 blank sample (without reaction reagent) were prepared along with saliva samples to provide a calibration curve. Standard glucose solutions were prepared by spiking pooled saliva with different glucose concentrations to eliminate a matrix effect. Samples were then mixed with reagents from glucose assay kits following the manufacturer's protocol and incubated at 37°C for 40 minutes. Absorbance measured at 570 nm wavelength was proportional to glucose levels. SG levels were then calculated using the calibration curve from this batch test.

According to results from healthy human subject research, the lowest SG level obtained was 1.1 mg/dL, while the highest was 10.1 mg/dL. However, since patients with diabetes' SG level can reach down to 0.3 mg/dL during hypoglycemia condition, and raise higher than 20 mg/dL during hyperglycemia condition, a broader SG measurement range is in need to be verified to warn patients from these dangerous situations. Also, a shelf life of at least 6 months is required to apply sensors in clinical exercise. In the future, rugged packaging needs to be designed to protect sensors and retain bioreactivity during storage, so as to achieve longer shelf life for such biosensors.

Now-a-days the glucometer used go behind invasive procedure. Hence there is an insistent need to reinstate invasive procedure by non-invasive procedure. Glucose series in saliva instead of puncturing the skin is to test in in [35]. The principle of intention behind the work is to establish electrochemical reactivity which helps to identify the blood glucose level of the patient. As the salivary glucose level is directly related to the blood glucose level, diagnosing the salivary glucose level blood glucose can also be established. The normal glucose range in saliva is 0.5 – 1.00 mg/100ml for the normal blood glucose range is 70 – 99 mg/dl. The average range of glucose in saliva is 1 mg/dl for blood glucose range 84.5mg/dl. So the value difference between

blood glucose and salivary glucose is 83.75 mg/dl. Hence by deducing the salivary glucose value of the patient with the average blood glucose level or by adding the patient's salivary glucose value to 83.75, the patient's blood glucose value can be estimated. The strip consists of some lysis enzymes that can react with saliva. The reaction between enzymes and saliva form hydrogen peroxide (H_2O_2). The measurement of hydrogen peroxide relays the value of salivary glucose intensity. At this instant the salivary glucose level can be differentiated with typical blood glucose range and hence the blood glucose range of the patient can be deliberated. The device requires an electrode that can measure the hydrogen peroxide value by passing current. A suitable preamplifier is also required for input current amplification. Finally, a digital display unit is used to dissect the values. This approach can also be used in real time processing of blood glucose non-invasively.

To provide accurate, low cost, and continuous glucose monitoring, disposable saliva nano-biosensor has developed in [36]. Excellent clinical accuracy was revealed as compared to the UV Spectrophotometer. By measuring subjects' salivary glucose and blood glucose in parallel, it was found that the two generated profiles share the same fluctuation trend but the correlation between them is individual dependent. There is a time lag between the peak glucose values from blood and from saliva. However, the correlation between the two glucose values at fasting is constant for each person enabling noninvasive diagnosis of diabetes through saliva instead of blood. Furthermore, a good correlation of glucose levels in saliva and in blood before and 2 h after glucose intake was observed. Glucose monitoring before and 2 h after meals is usually prescribed by doctors for diabetic patients. Although the correlation between blood glucose and salivary glucose profiles is highly individual dependent, there is a good correlation between glucose levels in saliva and in blood before. Thus, this disposable biosensor will be an alternative for real-time salivary glucose tracking at any time. Saliva test could improve diabetes control and treatment [23, 37].

9. Conclusions

Finger pricking as the main routine in these invasive techniques is troublesome for diabetic patients because it can lead to scarring, do not allow to make glucose measurements many times per day in order to control results of the treatment to be done cheaply and in noninvasive way. Saliva stabilizes the ecosystem of the oral cavity; and hence, it serves as a marker to timely discover the disease that further leads to more successful treatment, risk estimation, estimation of glucose level and it is a simple, noninvasive alternative to blood and urine tests. The most common method of SMBG involves finger, but, in recent years, saliva, tears, urine etc. have also Used today together with saliva and glucose sensors smartphones act as robust, inexpensive, miniaturized systems which can help achieve the goal of "personalized medicine" where a man can easily conduct the testing himself at home with limited training and also lead to in situ diagnosis in poor as well as remote areas devoid of conventional equipment and health facilities. Used today screen-printed saliva sensor chip is functionalized with single wall carbon nanotube (SWCNT). Note that Saliva and its components, such as proteins and peptides, can be used as indicators of general health to measure risks of developing certain diseases. Such diagnostic methods include that for lactate, cortisol and biomarkers for diseases such as cancer in non-invasive body fluids as well as diabetes, serum, etc. There are many effective saliva tests [23, 37].

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