

## Spectroscopic Study of Human Saliva Sample by ATR-FTIR Spectroscopy for Detection of Diabetes Mellitus

Tavishi Singh Student ts1370@srmist.edu.in 9176218810 <i>Department of Biomedical Engineering</i> <i>SRM Institute of Science and Technology</i> <i>(Deemed to be University u/s 3 of UGC Act, 1956)</i> Kancheepuram, Tamil Nadu, India	Dr. S.P. Angeline Kirubha* Assistant Professor angelins1@srmist.edu.in 9790848570 <i>Department of Biomedical Engineering</i> <i>SRM Institute of Science and Technology</i> <i>(Deemed to be University u/s 3 of UGC Act, 1956)</i> Kancheepuram, Tamil Nadu, India	Saswati Dhal Student <a href="mailto:sd5077@srmist.edu.in">sd5077@srmist.edu.in</a> 7010401525 <i>Department of Biomedical Engineering</i> <i>SRM Institute of Science And Technology</i> <i>(Deemed to be University u/s 3 of UGC Act, 1956)</i> Kancheepuram, Tamil Nadu, India
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**Abstract**— A huge number of individuals overall live with diabetes and a few millions lose their life from it every year. A non-intrusive, easy strategy for glucose testing would profoundly improve consistence and glucose control while lessening difficulties and the overall disease administration costs. To provide accurate, low cost, and continuous glucose monitoring, in this paper we have tried to test for alternative method by ATR-FTIR spectroscopic studies. The study was conducted on fifteen diabetic and ten healthy individual saliva samples. ATR-FTIR was carried out on the samples. For the validation purpose a standard fasting blood glucose measurement was taken. The correlation between blood glucose level and salivary glucose was established using the transmittance percentage.  $950\text{--}1180\text{ cm}^{-1}$  is considered as the spectral region of glucose or sugar moieties. A transmittance percentage peak was found at  $1045\text{cm}^{-1}$  for diabetic subject which shifted to  $1039\text{cm}^{-1}$  for non-diabetic subjects.

**Keywords**— *Diabetes Mellitus, Saliva, FTIR Spectroscopic Analysis, Glucose Monitoring, Non-invasive.*

### 1. INTRODUCTION

One of the medical ailment found ubiquitous is diabetes mellitus. The International Diabetes Federation has estimated about 463 million people of age group 20-79 years are diabetic in 2019 and it can reach around 700 million by 2045[1]. Diabetes mellitus is a type of metabolic disorder which is caused due to hyper glycaemia and insulin adequacy and it brings changes in blood glucose agglomeration whose normal range is in between 80-120mg/dL. Around the globe due to diabetes mellitus disability and elimination of numerous person happens. There were 4.2 million deaths in 2019 due to diabetes, which equals to one death every 7.5 second. Complications such as heart disease, circulatory problems, stroke, kidney failure and blindness can be circumvented by preliminary diagnosis, on-time treatment and uninterrupted monitoring of the disorder, which is indispensable to patient's life [1]. Monitoring of blood glucose is ongoing utilization for diabetes administration. The monitoring of blood glucose plays a vital role in deciding the insulin dose to be given and also in detection of abnormal

glucose concentration which can lead to dietary changes, adverse medication responses and prolonged illness. The inconvenience and exertion caused by finger-pricking are the major reason for dislike of these obtrusive tests which results in lesser test and meagre blood glucose control. Expensive administration costs and severe complications are the outcome of inconsistent blood glucose control. Specifically, in juveniles incessant agonizing finger pricks is a major obstacle and results in same pessimistic consequences for disease management. In 1841, an alternative method for glucose sensing was established by analysing urine, but infelicitously there was inconsistency in the correlation of plasma glucose and urine [2]. Blood glucose monitoring is exclusive concede and universally accepted technique. There are multiple kinds of blood glucose measuring devices available in retail; but pricking of the finger is necessary each time to obtain blood samples for monitoring. Different kinds of non-invasive or minimally invasive approach was considered for further studies, which included surface plasmon resonance, infrared (IR) spectroscopy, Raman spectroscopy and fluorescence. Nonetheless, the outcome of sensitivity and reliability was limited by skin thickness and signal-to-noise ratio and was not directly co-related by blood glucose quantification. For instance, a glucose measuring wearable gadget was launched by Cygnus Inc. in 2002 named GlucWatch which measured the glucose level electro osmotically extracted beyond the skin [3]. But in the device there was problem of sweat collection and the accuracy level was low which made it difficult to use resulting in its termination from the outlets. Orsense Limited also launched a product that used an optical method known as “occlusion spectroscopy,” for the detection of blood glucose agglomeration [5], which is still under update. Albeit optical advances for glucose assurance are accessible, the majority of them are for lab use because of the size, cost, and multifaceted nature of activity. Thus, there is a high insistence for a non-obtrusive, expedient, precise, simple to-utilize, versatile, and minimal effort analytic apparatus for administration of diabetes. According to Lei et al. [9], the three obligatory essentials for major clinical applications: (i) the biological samples should be collected by a simple and inexpensive method with negligible distress, (ii) the biomarker related with wellbeing or malady ought to be explicit, and (iii) the instrument utilized for infection analysis and wellbeing screening ought to be exact and convenient with simple to- utilize innovation. One of the enticing biomedium is saliva, often referred as ‘mirror of the body’ for clinical diagnosis. Its novel properties, for example, non-invasive availability and the nearness of copious sickness biomarkers, make it especially alluring for ailment detection and monitoring. Salivation can be effectively gathered by people with unassuming guidance and it significantly lessens the uneasiness of the diagnosis. Salivation changes are helpful in demonstrating health of an individual. Saliva contains enormous number of symptomatic analytes such as HIV immune response, glucose and steroid hormones. At the beginning thiocyanate particles levels were depicted in saliva in place of blood to distinguish between smokers and non-smokers. Saliva was perceiving most promising results, when the results of biomedica such as saliva, blood and urine was analysed. Oral malignancy was more precisely identified by saliva rather than blood. Moreover, some other biomarkers of infection that surpassed in blood have their centralization in saliva, making utilization of saliva a preferred biomarker for clinical diagnostics.

## 2. MATERIALS

### 1.1. Reagents used for sample preparation

Iso-Propyl Alcohol ( $\text{CH}_3\text{CH}(\text{OH})\text{CH}_3$ ) (Chenchems) was used to clean the instrument used to avoid any kind of alterations. 0.2 $\mu\text{L}$  mini start filter was used to filter the saliva sample. Cold pack (Alpha Aesar, A Thermo Fisher Scientific Company) was to temporarily store the collected saliva sample to avoid any kind of alterations.

### 1.2. Appratus used for analysis and validation

ATR-FTIR analysis was carried using germanium prism by FT-IR, SHIMADZU IR TRACER-100, IR REGION 400-12500  $\text{cm}^{-1}$ , standard KBr mode, ATR mode. ATR-FTIR analysis was carried out in Dr.C.V.Raman Research Park, Department of Chemistry, SRM Institute of Science and Technology .

Fasting blood glucose level of the subjects was measured using ACCU-CHECK ACTIVE, (Roche Diabetes Care India Private Limited), at the time of sample collection for further validations.

### 3. METHODOLOGY

#### 3.1. Study Population

The study was carried out on patients after signing the informed consent release. 15 patients suffering from diabetes mellitus and 10 healthy individuals were enrolled. The study population and the control group were both analyzed according to the patients' demographic determinations: age, gender, and weight.

Of the 20 diabetic patients, 11 were men (73.33%) and 4 were women (26.6%) with an average age of  $57 \pm 22$  years. They were suffering from diabetes by one year or more. The 10 healthy individuals consist of 7 males (70%) and 3 females (30%) with an average age of  $54 \pm 17$  years.

#### 3.2. Saliva Collection

The sample was collected in the morning for the diabetic and non-diabetic subjects with minimum of 8 hours of fasting. The subjects were asked to expectorate into a sterile polypropylene tube box and stored in cold packs in insulated packs. The sample were stored at  $-20^{\circ}\text{C}$  before analysis was performed. Subjects were requested not to drink (except for water), not to smoke not to use steroid, lotions or creams or chew gum, the night before saliva sampling. A total of thirty samples were collected; fifteen of Diabetic Subject and ten of control subjects. The control subjects were healthy individuals, were non-smokers and not associated with any diseases. No adverse effects associated with saliva sampling were reported.

#### 3.3. Infrared Saliva Spectroscopy

Saliva samples were analysed using infrared spectrometer (SHIMADZU IR TRACER-100) equipped with attenuated total reflectance (ATR) sampling accessory. Using ATR, the internal reflection of light generates an evanescent wave that penetrates the sample to a depth of  $0.5\text{--}2\text{ }\mu\text{m}$ , depending on the wavelength of the light, the angle of incidence, and the indices of refraction of the ATR crystal, but independent from sample thickness. ATR crystal is made of a high refractive index material, Germanium in our case. Consequently, this approach is particularly suitable for measuring substances displaying strong infrared absorption such as water, or water based biological samples such as saliva.



Figure 1. Prepared saliva samples for FTIR Analysis

The saliva sample was brought to liquid state from  $-20^{\circ}\text{C}$  and was filtered using  $0.2\text{ }\mu\text{L}$  mini start filter and stored in Eppendorf tubes for different analysis.  $7\text{--}10\text{ }\mu\text{L}$  saliva was taken from the filtered sample and stored in Eppendorf tube [Fig. 1] for 15 diabetic subjects and 10 control subjects. The samples were sent for ATR-FTIR Analysis.

The spectra are obtained in the wavenumber range between  $4000$  and  $650\text{ cm}^{-1}$  with spectral resolution of  $4\text{ cm}^{-1}$ . For each sample,  $5\text{ }\mu\text{L}$  of saliva was placed by pipette onto an ATR germanium

prism and 100 scans were made, using a signal acquired without sample background. This procedure was repeated four times for each sample.

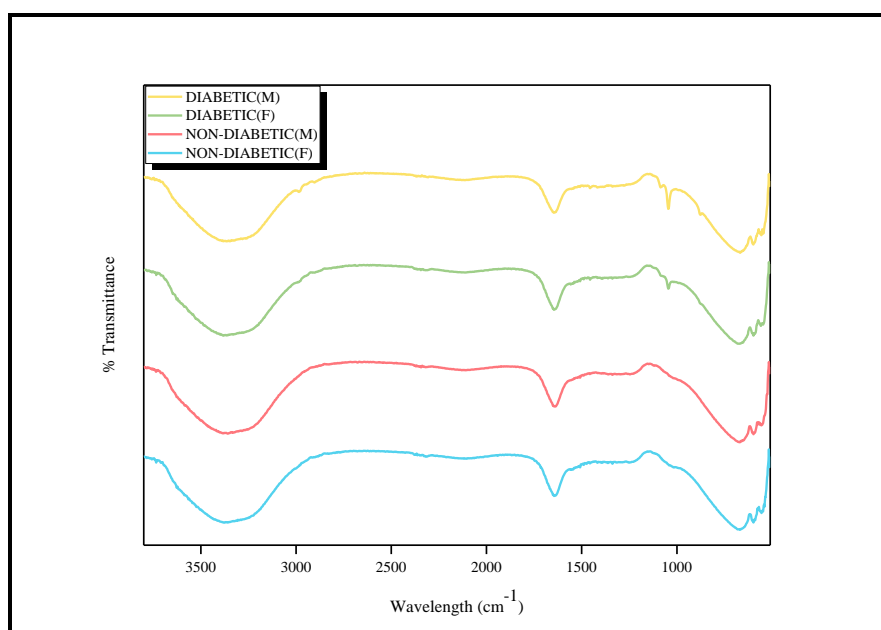
#### 4. RESULT AND DISCUSSION

All enrolled subjects affected by diabetes mellitus showed characteristic features on the IR spectrum. Diabetes associated signals that are represented by few particular molecules has been screened by utilizing ATR-FTIR Spectroscopy, for example, by-product of initial glycation and glucose.

The distinctive absorption profile of every sample in regards to different contributing spectra has completed the quantitative data. The fundamental initial step in recognition of potential protein biomarker of the ailment is by building a comprehensive catalogue of salivation proteins utilizing the proteomic approaches. The relative content of bio-molecules such as functions or the adjustment in the structure is changed due to the preceding of the any kind disease ailing in the body. The premier reason is the disturbances caused in the intermolecular or intramolecular interactions. The essential organic materials present in saliva are urea, uric acid, free amino acids, glycoprotein, mucin, carbonic anhydrase. The fig. 2 shows the spectra of diabetic and non-diabetic individuals. Protein constituent of the sample mostly dominates the characteristic vibrational mode. The vibrational band assignments were finished by the possibility of group frequencies of different analytes that are present in the example.

**TABLE 1. Vital information of subject used for ATR-FTIR Spectral saliva analysis**

SUBJECT NO.	AGE (Yrs)	SEX(M/F)	WEIGHT (Kgs)	YEARS OF DIABETES	FBGL (mg/dL)
1	55	F	69	-	104
2	54	M	69	-	85
3	52	F	45	1	245
4	45	M	76	1	356



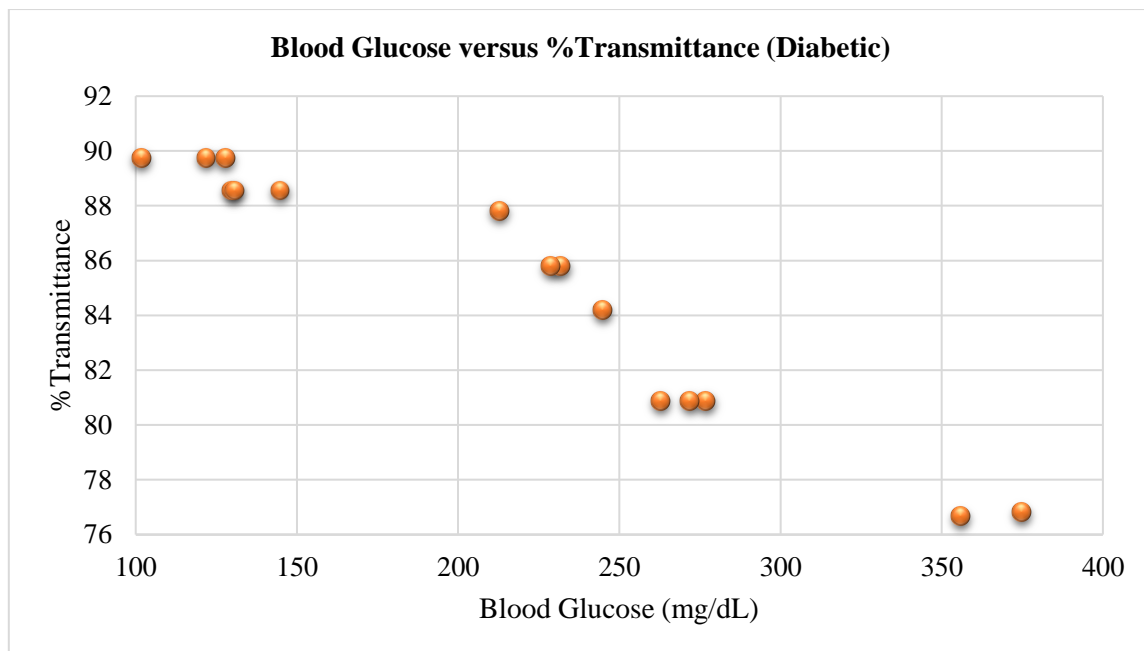
**Figure 2. ATR-FTIR Spectrum obtained for Diabetic and Non-Diabetic subjects**

The spectral region of 3600-3000  $\text{cm}^{-1}$  depicts the stretching vibration of proteins such as N-H, C-H and O-H [Fig. 2]. Due to C=O stretching the absorbance band is present in between 1600-1800 $\text{cm}^{-1}$  [Fig. 2]. The prominent absorption peak at 1646 $\text{cm}^{-1}$  is due to  $\text{NH}_2$  guanine, Amide-I C<sub>5</sub> methylated cytosine C=O stretching C=C uracyl [Fig. 2]. The peak at 1646  $\text{cm}^{-1}$  has confirmed the presence of C<sub>5</sub> methylated cytosine C=O stretching C=C uracyl,  $\text{NH}_2$  guanine. The spectral region of 1250-925  $\text{cm}^{-1}$  is occupied by various C-O-C symmetric and asymmetric vibrations done by phospholipids of proteins [Fig. 2]. 950-1180  $\text{cm}^{-1}$  is the spectral region of glucose or sugar moieties, the peak found at 1045  $\text{cm}^{-1}$  is due to presence of number of glucose molecules in diabetic subject but the peak shifted to 1039  $\text{cm}^{-1}$  for non-diabetic individual. The ( $\text{PO}_2^-$ ) symmetric phosphate stretching modes originate from the phosphodiester groups in nucleic acids and suggest an increase in the nucleic acid and C-N stretching absorption of aliphatic amines is weak which is depicted at the spectral band present at 1075  $\text{cm}^{-1}$  the spectral band present at 940  $\text{cm}^{-1}$  is due to carotenoid. The estimate of carbohydrate concentration is given by the peak present at 575 $\text{cm}^{-1}$  [Fig. 2].

The transmittance percentage of salivary glucose is inversely proportional to the level blood glucose in the blood. As the blood glucose increases the percentage transmittance of the salivary glucose decreases [ Fig. 3 and Fig. 4]. The maximum transmittance percentage for diabetic subject is 89.73028, for patient number 10 having fasting blood glucose of 102 mg/dL. The minimum transmittance percentage for diabetic subject is 76.660514, for patient number 15 having fasting blood glucose of 356 mg/dL [Fig. 3]. The peak transmittance for diabetic subjects is at 1045 $\text{cm}^{-1}$  [Fig. 2].

**Table 2. %Transmittance of Salivary Glucose in accordance to Blood Glucose for Diabetic subjects**

SUBJECT NO.	AGE (Yrs)	SEX (M/F)	WEIGHT (Kgs)	YEARS OF DIABETES	FASTING BLOOD GLUCOSE LEVEL (mg/dL)	% TRANSMITTANCE OF SALIVARY GLUCOSE
1	72	M	68	2	232	85.78273
2	68	M	72	3	229	85.78273
3	72	M	78	15	277	80.86834
4	74	M	91	2	272	80.86834
5	58	M	90	7	128	89.73028
6	55	M	80	1	263	80.86834
7	52	F	45	1	245	84.188763
8	55	M	62	0.5	122	89.7488
9	50	F	78	7	130	88.54125
10	70	M	80	5	102	89.73028
11	35	M	40	0.5	375	76.8164
12	43	M	65	9	145	88.54125
13	36	F	70	7	213	87.80834
14	74	F	45	16	131	88.54125
15	45	M	76	1	356	76.660514

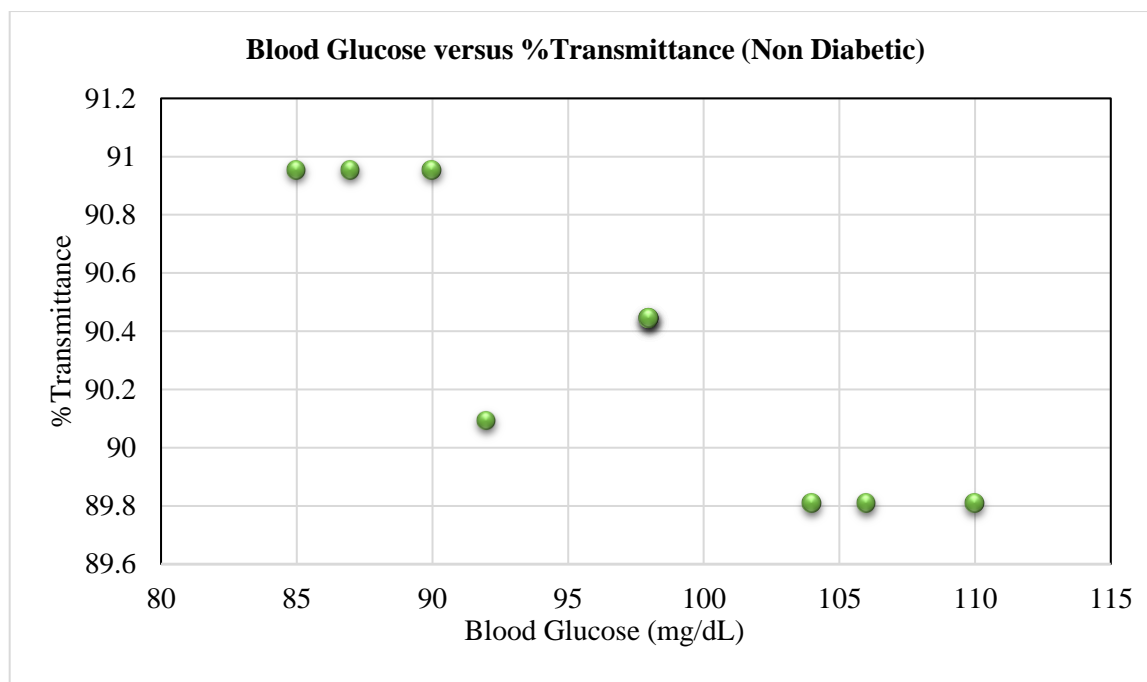


**Figure 3. Diabetic subjects showing %Transmittance of Salivary Glucose in accordance to Blood Glucose**

**Table 3. %Transmittance of Salivary Glucose in accordance to Blood Glucose for Non- Diabetic subjects**

SUBJECT NO.	AGE (Yrs)	SEX (M/F)	WEIGHT (Kgs)	YEARS OF DIABETIC	FASTING BLOOD GLUCOSE LEVEL (mg/dL)	% TRANSMITTANCE OF SALIVARY GLUCOSE
1	47	M	70	-	98	90.44371
2	59	M	72	-	90	90.95359
3	64	M	74	-	98	90.44371
4	47	M	80	-	98	90.44371
5	38	F	62	-	106	89.80838
6	72	M	77	-	110	89.80838
7	55	F	69	-	104	89.80838
8	54	M	69	-	85	90.95359
9	66	M	71	-	92	90.09242
10	44	F	65	-	87	90.95359





**Figure 4. Non-Diabetic subjects showing %Transmittance of Salivary Glucose in accordance to Blood Glucose**

The maximum transmittance percentage is 90.95359, for subject number 10 having fasting blood glucose of 87 mg/dL. The minimum transmittance percentage is 89.80838, for subject number 7 having fasting blood glucose of 104 mg/dL [Fig. 4]. The peak transmittance for non-diabetic subjects is at  $1039\text{cm}^{-1}$  [Fig. 2].

The spectral analysis shows there is no relation between the age, sex or weight of the subject. Hence, the inference is blood glucose level is inversely proportional to the transmittance percentage of glucose present in the saliva without any correlation of age, sex or weight of the individual.

## 5. LIMITATIONS

The estimations require a research facility as the sample have to be handled by trained experts because of their significant processing time, extortionate reagents and complex instrument. Subsequently, these techniques can't be utilized for continuous glucose monitoring at home. As of recently, there isn't appropriate technique for home consideration estimation of glucose utilizing saliva. Innovations, including microchips and micro-fluidic gadgets using FT-IR analysis, show incredible potential in building up a powerful, practical, precise, convenient, and simple to-utilize analytic device for saliva investigation. Scaled down saliva based analytic advancements will empower the utilization of follow measure of bio-fluids to give snappy and dependable outcomes for clinical dynamic and treatment results foreseeing. There is a time lag of few moments in the peak value of salivary glucose and blood glucose. Individual not suffering from any ailment was taken as control and validation to avoid any kind of outrageous detection of glucose concentration as well as diabetic individuals were not suffering from any other ailment than that of diabetes mellitus to avoid any variations in the analysis.

## 6. CONCLUSIONS

The ATR-FTIR Spectroscopic analysis was carried out and the absorbance is studied. In the analysis of saliva by non-diabetic and diabetic subjects, the importance of FTIR spectroscopy is clearly demonstrated both qualitatively and quantitatively. The intensity ratio parameter has been determined and it was discovered that the spectra of the diabetic saliva shows a greater absorption value than the spectra of the non-diabetic saliva because of glucose or sugar moieties present in the ailing saliva of the diabetic subjects. The validation of the spectral analysis was achieved by comparing it to blood glucose measurement taken at the time of sample collection. Miniaturized down salivation based analytic innovations will empower the utilization of trace amount of bio-fluids to give fast and solid outcomes for clinical diagnostics and treatment results anticipating. Numerous examinations conducted around the globe have divulged an optimistic correlation between the concentration of salivary glucose and blood glucose. The only parameter that was notably influenced during diabetes mellitus was the salivary glucose. That is why, for screening of undiagnosed diabetes and pre-diabetes and as strategy for diagnosis and monitoring of diabetes mellitus salivary glucose can be considered as an option.

## 7. FUTURE ENHANCEMENTS

Spectroscopy technique can be reliable source of glucose measurement. The devices can be miniaturized for home application. As saliva has the positive correlation with the blood glucose it can be used for continuous blood glucose monitoring by an individual with an ease. An optimistic association has been found between glucose content present in saliva and blood that can be used for screening of undiagnosed diabetes and pre-diabetes and as an alternative strategy for diagnosis and monitoring of diabetes mellitus. The non-invasive determination of diabetes by replacing blood glucose to salivary glucose indicates steady glucose level at fasting for every individual showing an optimistic result for determination of diabetes through saliva rather than blood.

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