Biosensors and Bioelectronics 123 (2019) 85–100

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Different strategies for detection of HbA1c emphasizing on biosensors and point-of-care analyzers

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ARTICLE INFO

Keywords:
HbA1c
Biosensors
Point-of-care
Diabetes mellitus
Chromatography

ABSTRACT

Measurement of glycosylated hemoglobin (HbA1c) is a gold standard procedure for assessing long term glycemic control in individuals with diabetes mellitus as it gives the stable and reliable value of blood glucose levels for a period of 90–120 days. HbA1c is formed by the non-enzymatic glycation of terminal valine of hemoglobin. The analysis of HbA1c tends to be complicated because there are more than 300 different assay methods for measuring HbA1c which leads to variations in reported values from same samples. Therefore, standardization of detection methods is recommended. The review outlines the current research activities on developing assays including biosensors for the detection of HbA1c. The pros and cons of different techniques for measuring HbA1c are outlined. The performance of current point-of-care HbA1c analyzers available on the market are also compared and discussed. The future perspectives for HbA1c detection and diabetes management are proposed.

1. Introduction

According to the definition given by World Health Organization, diabetes mellitus is defined as “a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion and/or insulin action” (Alanazi et al., 2017). Presently more than 220 million people have been diagnosed worldwide to be suffering from diabetes. This figure is far less than the actual number as people with impaired tolerance to glucose do not fall in this category (Gupta et al., 2017). Emergence of type 2 diabetes mellitus in children has risen due to number of factors such as lack of exercise, smoking, obesity, high blood pressure and high cholesterol levels. Nearly 90–95% cases of diabetes are type 2 diabetes mellitus and 50% people suffering from type 2 diabetes mellitus die due to cardiovascular diseases (Grundy et al., 1999). Chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart, and blood vessels (Association, 2008). The disease causes high levels of blood glucose as a result of defects in insulin production (type I), insulin action (type II), or both (Leslie et al., 2016). Chronically uncontrolled hyperglycemia leads to a wide range of adverse health outcomes including retinopathy, nephropathy, neuropathy, and cardiovascular disease (Collaboration, 2010). These complications results in significant mortality and morbidity for patients with diabetes (Lupsa and Inzucchi, 2018; O'Gara et al., 2012). Thus, there is high demand for development of tools for precision monitoring of diabetes and diabetes management.

Glucose levels in blood can be measured reliably, accurately and quickly, using any number of small, handheld devices available from pharmacies. But these tests provide a snapshot view of blood glucose only at the time of diagnosis. Although information provided by these tests can be useful for determining the correct amount of insulin to be injected, but it does not provide a long-term view that could be useful for managing diabetes (Gupta et al., 2017). This has unfolded paths for detecting blood glycated hemoglobin A (HbA1c) levels (Sherwani et al., 2016). The primary study documenting that knowledge of HbA1c values would result in improved metabolic control was published in 1990 (Larsen et al., 1990). It was concluded that regular measurement of HbA1c leads to changes in diabetes treatment and improvement of metabolic control, indicated by lowering of HbA1c values. The American Diabetes Association (ADA) in their 2003 consensus guidelines recommended that HbA1c testing should be performed routinely in all patients with diabetes (Foundation et al., 2003). However, the analysis of HbA1c tends to be complicated because HbA1c is formed by non-enzymatic glycosylation of hemoglobin. There are more than 300

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https://doi.org/10.1016/j.bios.2018.06.018
Received 16 April 2018; Received in revised form 23 May 2018; Accepted 6 June 2018
Available online 07 June 2018
0956-5663/

different assay methods of measuring HbA1c (Chandalia and Krishnaswamy, 2002). Many factors may interfere with HbA1c measurements according to assay method used, producing falsely high or low values. This review is to outline the current research activities on developing assays such as chromatographic techniques and biosensors for the detection of HbA1c in last five years. The pros and cons of different techniques for measuring HbA1c are outlined. The need for standardization of different assays is proposed and the current point-of-care HbA1c analyzers available in market are also compared.

2. HbA1c

Glycated hemoglobin is known as HbA1c, hemoglobin A1c, A1c, HGBA1c or HB1c. It is formed when hemoglobin is exposed to plasma glucose via a non-enzymatic pathway. In HbA1c one or both N-terminal valines of hemoglobin (Hb) are glycosylated (Lenters-Westra et al., 2013). It is a stable minor hemoglobin variant and represents 4–6% of total hemoglobin content blood of healthy adults (Liu et al., 2008). The use of HbA1c for monitoring the degree of control of glucose metabolism in diabetic patients was proposed in 1976 by Koenig and co-workers (Koenig et al., 1976). It gives the measurement of glucose concentration over a longer period of time (120 days) in diabetic patients (Gupta et al., 2017; Jain et al., 2017). The results obtained by HbA1c analysis are often reliable with few exceptions such as patients having fluctuations between very low and very high levels of glucose, and increased blood loss due to hemolysis or red cell disorders in uremic patients. This is so because all these situations lead to shorter lifespan of red blood cells (less than 120 days) and as glycation of hemoglobin occurs in 90–120 days, therefore, the results obtained in such conditions are misleading (Xanthis et al., 2007).

HbA1c was isolated using chromatographic column from other isomeric forms of hemoglobin in 1958 (Huisman et al., 1958) and characterized as glycoprotein in 1968 (Bookchin and Gallop, 1968). In 1969 it was found that patients suffering from diabetes have increased levels of HbA1c (Rahbar et al., 1969) while the glycation reactions between them were explained by mid 1970s (Bunn et al., 1975). Glycation of hemoglobin is a non-enzymatic process in which valine at β-globin chain of hemoglobin covalently binds with glucose forming aldimine linkage. The intermediate formed then goes through an Amadori reaction forming HbA1c (Fig. 1). The reaction takes place by the nucleophilic attack between the carbonyl group of glucose and amino group of hemoglobin forming Schiff's base. This reaction is reversible and takes several hours to complete but once Schiff's base is formed, it undergoes rearrangements to form glycated form of hemoglobin (Ansari and Dash, 2013) This glycosylated form persists until its death, and the ratio of HbA1c to hemoglobin (Hb) is a reliable indicator of average blood glucose level in a person. The results of HbA1c detection can assist diabetics, as well as their physicians, by providing treatment goals to reduce risks associated with development and progression of chronic complications of diabetes.

3. Analysis of HbA1c

Due to the high importance of HbA1c monitoring, more than 300 analytical methods have been developed for its analysis, such as immunoassay (Ang et al., 2016b; Siren et al., 2002), high pressure liquid chromatography (del Castillo et al., 2011; Hu et al., 2018; Lee et al., 2011), spectrophotometry (Adamczyk et al., 2006; Hirokawa et al., 2004; Syamala Kiran et al., 2010; Zhernovaya et al., 2008), ion-exchange and affinity chromatography (de la Calle Guntiñas et al., 2003; Li et al., 2002; Maleska et al., 2017; Thevarajah et al., 2009), electrophoresis (Hamer et al., 2018; Koval et al., 2011; Marinova et al., 2013), boronate affinity chromatography (Li et al., 2002; Pribyl and Skladal, 2005; Usta et al., 2016), colorimetric methods (Wangoo et al., 2010) and mass spectroscopy (Lee et al., 2005; Redman et al., 2016). Many point-of-care (POC) analyzers for measuring HbA1c are also available in the market. Selection of the method for HbA1c detection can be decided based on equipment availability, its cost and characteristics of population to be tested. In the below sections, we will discuss these assays according to chromatography techniques, biosensors and POC analyzers.

3.1. Chromatographic techniques for detection of HbA1c

3.1.1. Ion-exchange chromatography and high-performance liquid chromatography

Ion-exchange chromatography allows the separation of Hb species based on charge differences. When the cation-exchange column is subjected to different buffers of increasing ionic strength, Hb species elute from the column at different times, depending on their charge. The glycosylated forms are more negatively charged and elute first than the non-glycosylated ones in such columns. The concentration of Hb is measured after elution from the column, which is then used to quantify HbA1c by calculating the area under each peak (Bry et al., 2001). Therefore, high performance liquid chromatography (HPLC) in combination with other techniques has been widely used to detect HbA1c levels in number of patients.

The glycohemoglobin analyzer is an instrument used for measuring HbA1c % in blood. It provides excellent reproducibility and has been regarded as the designated comparison method in national glycohemoglobin standardization program. Its mode of operation is based on both HPLC and cation-exchange chromatography. In a study conducted

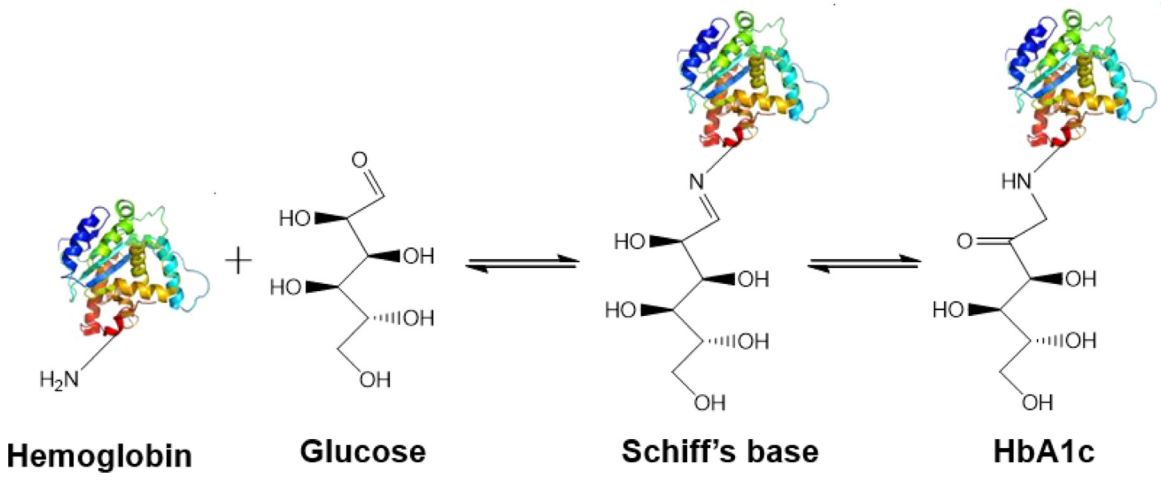

Fig. 1. The process of HbA1c production by glycation of hemoglobin.

by Out Patients Department, Universal College of Medical Sciences Teaching Hospital, Bhairahawa, Nepal it was found that ion exchange chromatography could save time by having reports ready at the time of patients visit to physicians (Gautam et al., 2014). Capillary Electrophoresis (CE)-HPLC was used to evaluate HbA1c levels in a pregnant woman and 5.4% HbA1c could be detected in blood along with some Hb variants (HbA2 and HbA0) (Zhong et al., 2016). HPLC combined with liquid chromatography-tandem mass spectrometry (LC/MS/MS) was used to detect HbA1c levels and results were compared with the reference measurement procedure reported by International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) (Zhang et al., 2016). The HbA1c levels from 30.4 to 145.8 mmol mol^{-1} were detected which were found to be very reliable and this method was accepted by IFCC for clinical measurements. An automated HPLC was used for detection of HbA1c levels in dried blood samples and it was found that the results were comparable to those obtained in whole blood samples (Maleska et al., 2017). The results obtained were validated under ISO15189 guidelines which proved it to be useful tool for diabetes management. However, use of HPLC suffers some disadvantages, such as requirement of a specialized HPLC analyzer system, a long run time, limitations of processing capability, and highly technical maintenance (Thevarajah et al., 2009). In addition, sometimes it provides inaccurate results due to other forms of Hb interferences, mainly coming from Hb carbamylation (described in uremic patients) or acetylation (due to the presence of high doses of salicylic acid) that must be considered (Maleska et al., 2017). In recent years, HbA1c measurement techniques other than HPLC method have been developed to solve the above-mentioned issues.

3.1.2. Boronate affinity chromatography

The separation and analysis of analytes using boronate affinity chromatography is carried out based on biological interactions within the given sample. Small saccharide molecules like glucose and fructose can be detected using boronate affinity chromatography (Kong et al., 2014; Sun et al., 2014; Torul et al., 2014) as these molecules form boronate esters with phenylboronic acid (PBA) (Li et al., 2015; Tabatabaei et al., 2016). Unique and stable cis-diol configurations are found by binding of glucose to hemoglobin to which boronate binds and can be traced easily. The value calculated using this method is known as true or total HbA1c and patient's value is expressed as % HbA1c of this total HbA1c. The detection range of this method is 5.3–17% (Gupta et al., 2017). Boronate affinity chromatography can be used to distinguish between Hb and HbA1c by specific binding to PBA (Usta et al., 2016). Monodispersed, magnetic polymethacrylate microspheres shell coated with silver nanoparticles (AgNPs) and PBA were used for boronate affinity chromatographic determination of HbA1c. Glycosyl residues of HbA1c forms cyclic boronate ester by binding to AgNPs functionalized with p-aminothiophenol (PATP). HbA1c analysis was carried out in HPLC systems and the values were used to prepare Surface Enhanced Raman Spectroscopy (SERS) calibration curves for HbA1c determination. Laser irradiation of 785 nm was used to illuminate PATP which gave direct indication of HbA1c (Fig. 2). The detection limit of this assay was 50 ng/mL (Usta et al., 2016). In another study, the electrode was modified with ferrocene-boronic acid (FcBA). FcBA binds specifically to HbA1c and not to non-glycated Hb as boronic acid derivatives can easily bind the sugar moiety of HbA1c. The levels of HbA1c can be detected by measuring the amount of FcBA eluted (Lin and Yi, 2017). Although, these chromatographic techniques give quite reliable and accurate response, they are time-consuming and expensive. Additionally, they require skilled personnel and sophisticated instruments, and can give false results due to the coexistence of genetic variants and other chemically modified derivatives of hemoglobin (Ang et al., 2015; Liu and Crooks, 2012; Pundir and Chawla, 2014; Shumyantseva et al., 2014). Therefore, it is desirable to develop cost effective, easy to use, robust and rapid methods for HbA1c detection (Ang et al., 2016a).

3.2. Biosensors for detection of HbA1c

A biosensor is an analytical device composed of an analyte, a transducer and physicochemical detector. The physicochemical detector may be an enzyme, nucleic acid, antibody or some microorganism (Banica, 2012). The transducer used may be optical, electrochemical, piezoelectric or electroluminescent depending on the interacting mode of analyte and physicochemical sensing element (Cavalcanti et al., 2008). The selection of proper receptor molecule, transducer and immobilization method is crucial in development of a biosensor. They help in selective, rapid, specific and cost-effective detection of the analyte (Gupta et al., 2017; Lin and Yi, 2017). These biosensors can support other detection methods for HbA1c such as isoelectric focusing, bioassay, ionophoresis, HPLC and ion exchange chromatography (Ahn et al., 2016). Fabrication of a multifunctional sensing interface is essential for a successful biosensor (Cao et al., 2017; Liu et al., 2016). A very thin conductive film having high surface area is preferable during the development of interface as it will provide fast response by keeping the sensing elements and biomolecules in close proximity (Qi et al., 2017). Additionally, the film should also be robust and remain intact during the sensing and washing steps. Also, it should have versatile chemistry for facile bioconjugation and applicable to wide array of analytes. Use of different nanocomposites can help in attaining interfaces possessing all these requirements such as increasing the sensitivity A number of nanocomposites such as silver and gold nanoparticles (AuNPs) (Goudarzi et al., 2016; Qi et al., 2016a), TiO_2 nanotubes (Jain et al., 2016; Zhao et al., 2016), multiwalled carbon nanotubes (MWCNTs) (Moon et al., 2017), graphene oxides (Qi et al., 2016b) and pyrroloquinoline quinine (PQQ) (Zhou et al., 2014b) have been used for the construction of biosensors. We have developed a HbA1c biosensor based on AuNPs modified sensing interface with the sensitivity of 0–23.3% (Liu et al., 2012a). According to the different transducers, different types of biosensors have been developed for the detection of HbA1c. The following sections will discuss these biosensors which are classified as electrochemical, optical, and colorimetric biosensors (Table 1).

3.2.1. Electrochemical biosensors for HbA1c detection

Electrochemical biosensors are the devices which can convert electrochemical signal into analytical signal (Yazdanpanah et al., 2015). The electrochemical biosensors are very popular for detection purposes because they have number of advantages such as high sensitivity, the simplicity of instrument, and miniaturisation. These biosensors can be developed by modifying electrodes based on boronic acid-diol recognition for HbA1c detection as under weak alkaline conditions boronic acid binds to sugar (via cis-diol group) on the surface of glycated proteins (Zhao et al., 2016). Based on the difference in transduction the electrochemical biosensors can be classified as potentiometric, voltammetric/amperometric, and impedometric biosensors (Wang and Anzai, 2015). Different electrochemical biosensors used for the detection of HbA1c are described as below.

3.2.1.1. Amperometric biosensors. Amperometric biosensors are the devices which measure the change in current due to oxidation and reduction of electroactive species in a biochemical reaction at constant potential whereas voltametric measures the change in current at variable range of potential (Grieshaber et al., 2008). Variable amperometric biosensors have been constructed for detection of HbA1c and they have proved to be very simple, fast and specific but they all require a specialized redox probe as HbA1c is electrochemically inactive. An amperometric biosensor for detection of HbA1c was fabricated using cellulose membrane modified with haptoglobin (group of serum proteins) which could bind with HbA1c on the electrode surface (Nielsen et al., 2013). Label-free sensors were also constructed using glassy carbon electrodes modified with ferrocene (Fc) along with AuNPs (Liu et al., 2013a) (Fig. 3) or monolayers of oligo

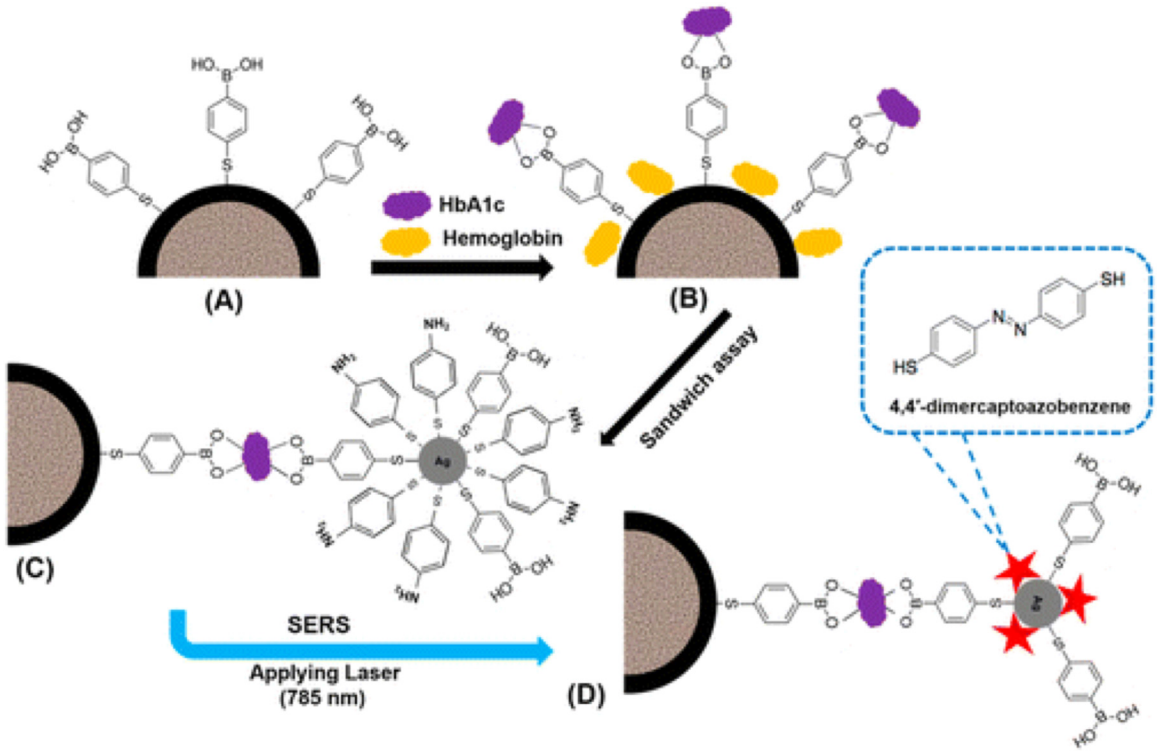

Fig. 2. Separation and detection of HbA1c using boronate affinity chromatography (A) Binding of MPBA to AgNPs, (B) Ester formation of HbA1c and MPBA, (C) Sandwich assay and irradiation with laser, (D) Measurement of photocoupling product and HbA1c levels using SERS. Reprinted with permission (Usta et al., 2016).

Table 1
Different biosensors for HbA1c detection.

Biosensors	Transducers	Detection range	References
Amperometric	AuNPs + aryl diazonium salt modified glassy carbon electrode	0–23.3%	(Liu et al., 2012a)
	Glassy carbon electrode + Gold nanoparticles + Glycosylated pentapeptide	4.6–15.1%	(Liu et al., 2013a)
	Gold electrodes modified with PBA	4.5–15%	(Song et al., 2013)
	Screen printed electrodes modified with pTTBA/AuNps	0.1–1.5%	(Kim and Shim, 2013)
	PBA-PQQ nanoparticles on electrode	9.4–65.8 μg mL^{-1}	(Zhou et al., 2015)
	Poly(3-aminophenylboronic acid) nanoparticles on electrode	0.175–2.808 μg mL^{-1}	(Wang et al., 2015)
	Gold nanoaprticles (GNPs), TiO_2 nanotubes and 12-phosphotungstic acid	0.009–36 μg mL^{-1}	(Jain et al., 2016)
	Titania nanotubes modified with gold nanoparticles and fructosyl amino acid oxidase	7.2×10^{-5}–12.96×10^{-3} μg mL^{-1}	(Zhao et al., 2016)
	pTBA or 2,2':5'5''-Terthiophene-3'-p-benzoic acid) and TBO (Toluidine blue O) on MWCNTs	1.08×10^{-4}–13.32×10^{-3} μg mL^{-1}	(Moon et al., 2017)
	Nitrogen doped-graphene nanosheets (GNs), gold nanoparticles (AuNPs) and fructosyl amino oxidase (FTO)	0.0054–36 μg mL^{-1}	(Jain and Chauhan, 2017)
	Graphene oxide + PBA-PQQ (phenylboronic acid-modified pyrroloquinoline quinine)	9.4–65.8 μg mL^{-1}	(Zhou et al., 2014b)
	Gold film coated with MPA-SAM	7.5–20 μg mL^{-1}	(Molazemhosseini et al., 2016)
	Au electrode, MWCNTs, graphene oxide (rGO), platinum nanoparticles (PtNPs) and fructosyl amino oxidase	0.0009–18 μg mL^{-1}	(Jain et al., 2017)
Potentiometric	MEM electrodes modified with AuNPs SAM	0.05–0.1705 μg mL^{-1}	(Xue et al., 2011)
	ISFET gate and electrode modified with poly(pyrrole) nanoparticles	6×10^{3}–1.71×10^{2} μg mL^{-1}	(Liu and Crooks, 2012)
	Screen printed carbon electrodes modified with polyethylene terephthalate (PET) film	5.6–10.6%	(Tanaka et al., 2017)
Impedometric	Thiopheneboronic acid coated parallel electrodes	0.01–0.1 μg mL^{-1}	(Chuang et al., 2012)
	Ring shaped interdigited gold electrodes with SAM of thiophene-3-boronic acid	1–15%	(Hu et al., 2014)
	Eggshell membrane modified with 3-aminophenylboronic acid	2.3–14%	(Boonyasit et al., 2015)
	Nitrogen-doped graphene/AuNPs/fluorine-doped tin oxide	10–65 μg mL^{-1}	(Li et al., 2017)
Optical	Upconversion nanoparticles (UCNPs) + Nanoarray of $NaGdF_4$:48% Yb^{3+}, 2% Er^{3+}	5.6–11.5%	(Hu et al., 2016b; Jo et al., 2016)
	CdTe QDs + anti-HbA1c antibodies	4–6%	(Gautam et al., 2014)
	Magnetic beads based on chip aptamer sandwich assay	7×10^{3}–21×10^{3} μg mL^{-1}	(Li et al., 2016)
	Carboxy-EG6-undecanethiol SAM + 3-aminophenyl boronic acid + Au film	2.5–17%	(Ahn et al., 2016)
Colorimetric	Secondary polyclonal rabbit anti-goat antibody + polyclonal anti-Hb antibody and monoclonal anti-HbA1c antibody	4.0–7.0%	(Ang et al., 2016a)
	Secondary polyclonal rabbit anti-goat antibody + polyclonal anti-Hb antibody and monoclonal anti-HbA1c antibody (Gold functionalized)	4–14%	(Ang et al., 2016b)
	AuNPs + anti-HbA1c antibody	1–4 μg mL^{-1}	(Wangoo et al., 2010)

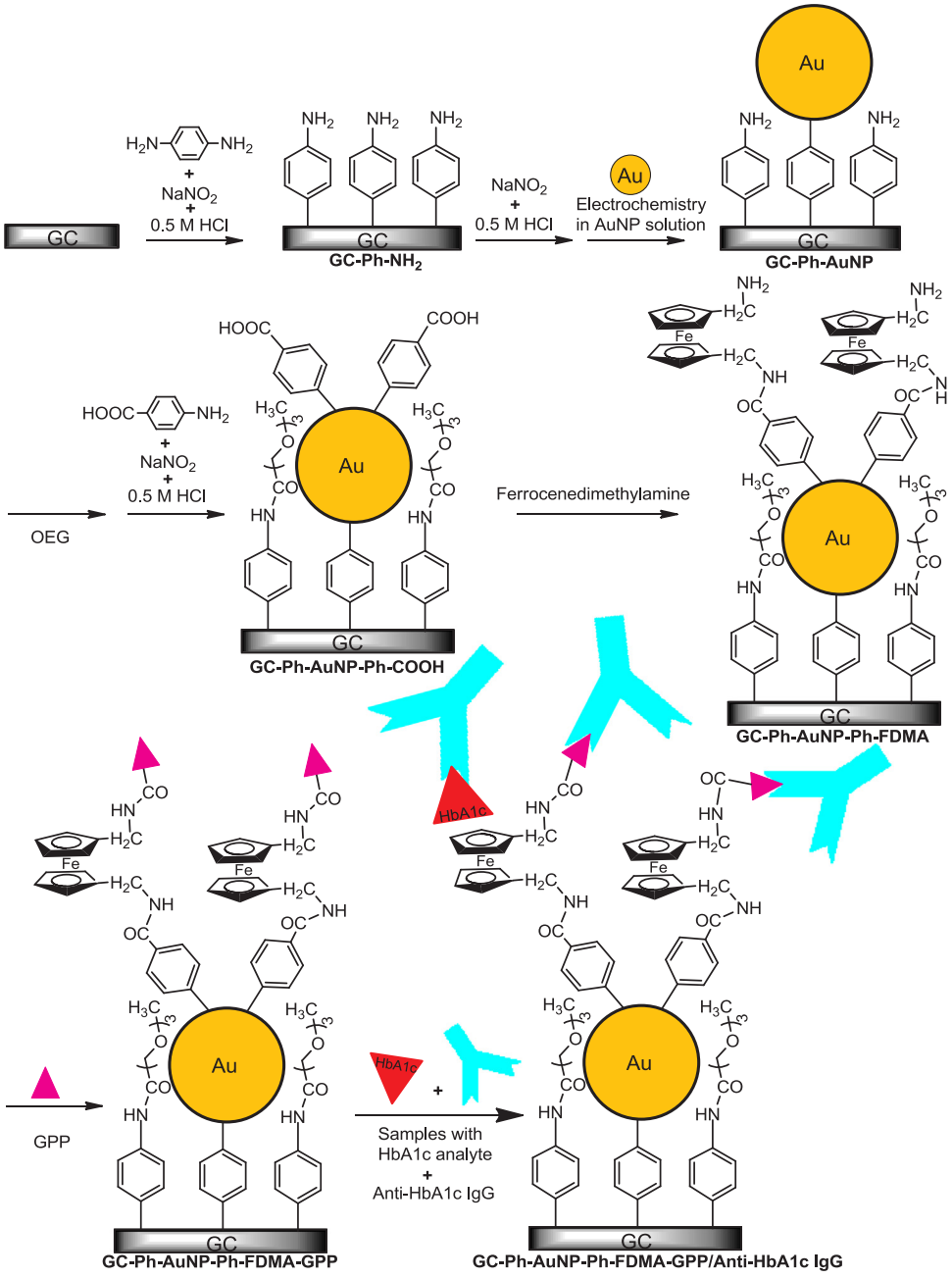

Fig. 3. Amperometric biosensor for HbA1c detection. Reprinted with permission (Liu et al., 2013a).

(phenylethynylene) molecular wire (Liu et al., 2012b). The introduction of AuNPs to the sensing system has significantly increased the sensitivity by providing big surface area to attach anti-HbA1c antibodies. Upon binding of anti-HbA1c antibodies to Fc moieties modified with glycosylated penta-peptide redox signals at 0.3–0.4 V were generated which decreased with time (Liu et al., 2013a, 2012b). Thus the modulation of Fc current can be used for quantification of HbA1c. Ferrocene derivatives are stable and experiences diverse range of structural versatility and thus, are preferred for the construction of label-free sensors for HbA1c detection (Takahashi and Anzai, 2013). Non-enzymatic biosensors for HbA1c detection can also be built as HbA1c catalyzes the reduction reaction which generates the current that could be detected amperometrically and results obtained are proportional to the HbA1c concentration. Based on this fact, a disposable amperometric biosensor was developed using PBA-appended poly(thiophene)/Au nanoparticles (pTTBA/AuNPs) immobilized on screen printed electrodes. Then aminophenyl boronic acid (APBA) was immobilized on the electrodes for selective binding of HbA1c. The complex formed reduced H_2O_2 which was detected amperometrically giving indication of HbA1c levels. The developed biosensor could be directly used for determining HbA1c levels in finger prick samples with the linear range of 0.1–1.5% and detection limit of 0.052 ± 0.02% (Kim and Shim, 2013). A regular check is recommended while operating with biosensors constructed using PBA as H_2O_2 oxidizes carbon-boron bond of PBA yielding mixture of phenol derivatives (Sato et al., 2014; Song et al., 2013).

Moreover, the sensing interface of biosensors need to be stable over longer period and also are applicable for direct detection of HbA1c in matrix samples such as blood, urine, or saliva (Zhou et al., 2014b). To meet these targets an amperommetric biosensor was constructed using glassy carbon electrodes coated with reduced graphene oxide and PBA-modified pyrroloquinoline quinine (PQQ). PQQ is a compound with

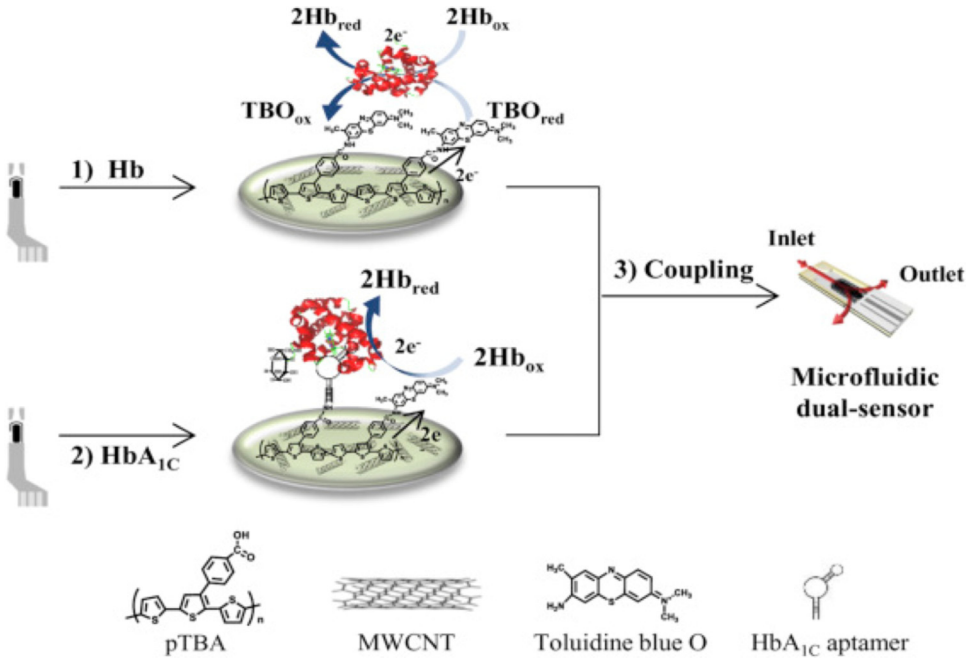

Fig. 4. Fabrication of dual electrode sensor for detection of Hb and HbA1c. Reprinted with permission (Moon et al., 2017).

heterocyclic o-quinone structure which catalyzes the transfer of electrons from quinine to quinol (Young et al., 2013). When PQQ was immobilized on electrode it could show similar transfer of electrons as it could do in its native state, therefore, PQQ could be used for development of electrochemical biosensors for detection of HbA1c. PQQ has been tailored on electrodes modified with carbon nanotubes (Kanninen et al., 2010) or graphene out of which latter produces better results due to its greater surface area (Chen et al., 2012; Zhou et al., 2014b). Core-shell magnetic nanoparticles have been used for the construction of electrochemical biosensors used for HbA1c detection. The electrodes are modified with different materials such as boronic acid, binding proteins and antibodies which have affinity for sugar (Molazemhosseini et al., 2016; Sridevi et al., 2016). When HbA1c reacts with PQQ on the electrode it forms boronic acid-diol which could be detected by measuring the changed oxidation peak current. The detection limit of this biosensor was 1.25 μg mL^{-1} whereas its detection range was 9.4–65.8 μg mL^{-1}. Moreover, the biosensor did not require chromatographic separation, and was compatiable with commercially available, non-biological, cheap materials and simple equipment (Zhou et al., 2014b).

Some other label-free biosensors have also been fabricated using PBA modified PQQ (Zhou et al., 2015) or poly(3-aminophenylboronic acid) nanoparticles modified electrodes (Wang et al., 2015). It was found that peak current generated was inversely proportional to HbA1c concentration in both biosensors. The latter biosensor had higher selectivity for HbA1c over glycated proteins such as serum albumin and blood sugars like galactose, mannose and glucose and did not interfere with redox potential and diffusivities of ions (Wang et al., 2015). A biosensor was constructed with gold film (10 nm) as working electrode and Ag/AgCl as reference electrode for the detection of HbA1c levels using differential pulse voltammetry. Anti-HbA1c antibodies were immobilized on gold electrodes using 3-mercaptopropionic acid-self assembled monolayer (MPA-SAM). The developed biosensor was disposable with the detectable range of 7.5–20 μg mL^{-1} HbA1c. It was cost effective and very useful in measuring HbA1c levels to monitor and control diabetes in patients (Molazemhosseini et al., 2016).

In order to increase the sensitivity of the sensing interface, nanomaterials such as TiO_2 nanotubes with cylindrical geometry are able to provide better biocompatibility and high surface area (Feng et al., 2014; Gao et al., 2014) which make them stable matrix for immobilization in comparison to spherical nanoparticles (Liu et al., 2013a). AuNPs could also be used in construction of biosensors as they provide support for molecules and also act as a bridge for electron transfer (Chauhan et al., 2013). An electrochemical biosensor based on nanocomposites of AuNPs and TiO_2 nanotubes was developed using 12-phosphotungstic acid as a reducing agent and electron mediator to transfer electrons between protein and conductor (Jain et al., 2016). The developed biosensor could detect HbA1c within 0.5–2000 μM with detection limit of 0.5 μM. The response time of biosensor was 3 s and gave reproducible results for 4 months. Another biosensor based on nanocomposites of titania nanotubes and AuNPs was fabricated using fructosyl amino acid oxidase immobilized on idium tin oxide glass. It could detect HbA1c from 0.004 to 0.72 μM. The detection limit of biosensor was 3.8×10^{-9} M and the biosensor was disposable, highly sensitive, low cost and gave results comparable to standard methods (Zhao et al., 2016). MWCNTs are developed using graphene which also serve to be a material of choice for development of biosensors with increased sensitivity (Subrahmanyam et al., 2010). It has extraordinary mechanical, structural and physical properties (Xia et al., 2015) due to which it provides great water absorption ability, large surface area and biocompatibility (Chauhan et al., 2015, 2016). Using the chemical methods MWCNTs can be conjugated to metallic nanoparticles where they provide an excellent support to the nanoparticles and provide them with larger surface area and better properties (Shi et al., 2012; Wang and Bi, 2014). A highly selective, sensitive, cost effective and stable biosensor was developed for detcetion of Hb and HbA1c simultaneously in finger prick blood samples (Moon et al., 2017). This biosensor comprised of toluidine blue O (TBO) and aptamer/TBO immobilized on MWCNT composite layers and conducting polymer thus, forming a microfluidic dual sensor with two electrodes facing each other in a microfluidic system. First the screen printed electrodes were modified via electropolymerization of MWCNTs and conducting polymer (pTBA or 2,2':5'5''-Terthiophene-3'-p-benzoic acid) and then TBO was immobilized on the electrodes (Fig. 4). The developed biosensor was characterized, optimized and then used to detect Hb and HbA1c concentration. It was found that detection range for Hb was 0.1–10 μM with detction limit of 82 (± 4.2) nM whereas for HbA1c the detection range was 0.006–0.74 μM and detection limit was 3.7

(± 0.8) nM (Moon et al., 2017).

Nitrogen doped-graphene nanosheets, AuNPs and fructosyl amino oxidase (FTO) enzyme was used in fabrication of an amperometric biosensor for HbA1c detection (Jain and Chauhan, 2017). The enzyme was immobilized using cross-linking method which provided the working electrode great stability. The detection range of biosensor was 0.3–2000 μM whereas its detection limit was 0.2 μM. The developed biosensor was stable up to 4 months and free from interferences caused by urea, bilirubin, uric acid, urea and ascorbic acid. In addition, an ultrasensitive amperometric biosensor was developed using Au electrode, MWCNTs, graphene oxide, platinum nanoparticles and fructosyl amino oxidase enzyme (Jain et al., 2017). Fructosyl amino oxidase enzyme digests HbA1c in samples at N-terminal amino acids, either valine or histidine. This results in the release of H_2O_2 which can be detected at electrode surface. The concentration of H_2O_2 released is directly proportional to HbA1c levels in the sample. The developed biosensor had response time of 3 s, detection range of 0.05–1000 μM and detcetion limit 0.1 μM. The developed biosensor was cost effective, sensitive, selective, precise, reliable and gave stable responses up to 12 weeks.

3.2.1.2. Potentiometric biosensors. Potentiometric biosensors are devices used to measure the accumulated charge potential at the surface of working electrode at zero current between the working and reference electrodes. In other words, potentiometric biosensors give the indication of ion activity in an electrochemical reaction (Grieshaber et al., 2008). Potentiometric biosensors for HbA1c detection were fabricated using anti-HbA1c antibodies conjugated to extended gate ion-selective field effect transistors (Bian et al., 2011; Xue et al., 2012). The electrodes were modified with poly(pyrrole)-Au composites to get improved response which can be detected in differential mode. The effects caused by adsorption of non-specific proteins were compensated by subtracting the reference gate potential from antibody-immobilized gate potential. Alizarin red S (ARS) could be used as an indicator for redox reactions in potentiometric biosensors (Liu and Crooks, 2012). Using this biosensor HbA1c levels could be detected in blood hemolysate samples. Initially, the potential of ARS-PBA complex was negative, but it got shifted towards positive when HbA1c was bound to the complex. This change in potential gave the indication of HbA1c concentration. The potentiometric measurements could be conjugated to optical and fluorometric determination to get improved sensors. One such biosensor was constructed using CdTe quantum dots conjugated to HbA1c (Wangoo et al., 2010). Both zeta potential and color change were measured to detect HbA1c levels. The major advantage of this biosensor was that only 1 μL of blood sample was needed (Chopra et al., 2013).

A micro immunosensor was developed by immobilizing AuNPs with mixed SAMs on micro-electro-mechanical-system electrodes and ion-sensitive field-effect transistor (ISFET) integrated chips which provided uniform distribution of AuNPs and low signal to noise ratio. The anti-HbA1c antibody was immobilized on the immunosensor and HbA1c levels were detected potentiometrically with detection range of 50–170.5 ng mL^{-1} (Xue et al., 2011).

3.2.1.3. Impedometric biosensors. When some protein binds on the surface of electrode the electron transfer is hindered which produces change in impedance. This principle is used in construction of impedometric biosensors for the detection of HbA1c. An impedometric biosensor was constructed using polydimethylsiloxane chamber and thiopheneboronic acid coated parallel electrodes and it could detect HbA1c levels in 10–100 ng mL^{-1} sample (Chuang et al., 2012). HbA1c forms boronate esters with PBA-graphene oxide and increased impedance could be observed as HbA1c concentration increased from 2.4 to 12 μM. Ring shaped interdigitated electrodes and microfluidic devices with parallel electrodes could also be used for fabrication of HbA1c sensors. The ring shaped interdigital gold electrodes were modified with SAMs of thiophene-3-boronic acid to develop a biosensor which could detect HbA1c levels (1–15%) (Hsieh et al., 2013; Hu et al., 2014). Another impedometric biosensor was developed by cis-diol interaction of HbA1c with eggshell membrane modified with 3-aminophenylboronic acid. The detection range of biosensor was 2.3–14% HbA1c and detction limit 0.19% (Boonyasit et al., 2015). A biosensor with linear range of 10–65 μg mL^{-1} and detection limit 1.70 μg mL^{-1} was fabricated using fructosyl amino acid oxidase immobilized on functionalized glass electrodes. The glass electrodes were modified using nitrogen-doped graphene/AuNP/fluorine-doped tin oxide and the biosensor was highly selective and sensitive for HbA1c (Li et al., 2017). Jain and Chauhan (2017) developed an impedometric biosensor for the detection of HbA1c by immobilizing thiophene-3-boronic acid (T3BA) self-assembled monolayer on the surface of gold electrodes. The sinusoidal voltage is applied at constant frequency forming loop due to electric field from which impedance can be calculated based on Ohm's law. The decrease in impedance is observed on esterification of HbA1c with T3BA which gives the indication of HbA1c levels in samples (Jain and Chauhan, 2017).

3.2.2. Optical biosensors for HbA1c

Optical biosensors are the devices with a biorecognition sensing element like antibodies, enzymes, nucleic acids, tissues, receptors and whole cells and an optical transducer system. The reaction of biorecognition sensing element with an analyte produces an optical signal which is directly proportional to the concentration of analyte. Optical biosensors help in real-time, direct and label-free detection of analytes with high sensitivity and selectivity. Optical biosensors could be used as a rapid, robust and sensitive tool for detection of HbA1c (Kwak et al., 2018). An optical biosensor based on upconversion nanoparticles (UCNPs) was developed for detection of HbA1c (Hu et al., 2016b). UCNPs are inorganic nanocrystals doped with lanthanide which can easily emit visible light of higher energy by converting infrared photons of lower energy (Gai et al., 2013; Liu et al., 2013b; Zhou et al., 2014a). As the infrared light is used as excitation source fluorescent signals generated are free of background signals (Hu et al., 2016a; Xiaofeng et al., 2016). Moreover, the signals generated have narrow emission bands and good photostability (Haase and Schäfer, 2011). The concentration of HbA1c is inversely proportional to the luminescence produced by UCNPs (Jo et al., 2016). A biosensor was developed consisiting of nanoarray of $NaGdF_4$:48% Yb^{3+}, 2% Er^{3+} which acted as the fluorescence donor and HbA1c as the acceptor. Luminescent Resonance Energy Transfer (LRET) was used to study the concentration of HbA1c. UCNPs emit green light upon excitation with light of 980 nm but when HbA1c binds to these UCNPs the green light is quenched which could be measured (Fig. 5). The developed biosensor had the detection range of 5.6–11.5% (Jo et al., 2016). Using the similar principle another biosensor was developed consisiting of an array of UCNPs for HbA1c detection. The photons are collected on surface of fabricated array and at the surface they are more sensitive towards the increasing concentration of HbA1c. Therefore, the developed biosensor had four times sensitive results as compared to direct detection and could be used repeatedly for 20 times (Hu et al., 2016b).

An optoelectrochemical biosensor was fabricated using water soluble and highly luminescent CdTe QDs conjugated with anti-HbA1c antibodies. The secondary antibodies were labeled with QDs which yielded both electrochemical as well as fluorescent signals. The HbA1c in normal human samples was 4–6% and higher in those suffering from diabetes. It was found that correlation of 96% for electrochemical and 89% for fluorescence was observed when compared with standard methods which indicates that this is a reliable method for HbA1c detection (Chopra et al., 2013).

Since the antibodies used in sensors are sensitive to change in pH and their high cost there is high demand to have alternative recognition molecules for developing new methods of HbA1c detection (Chang

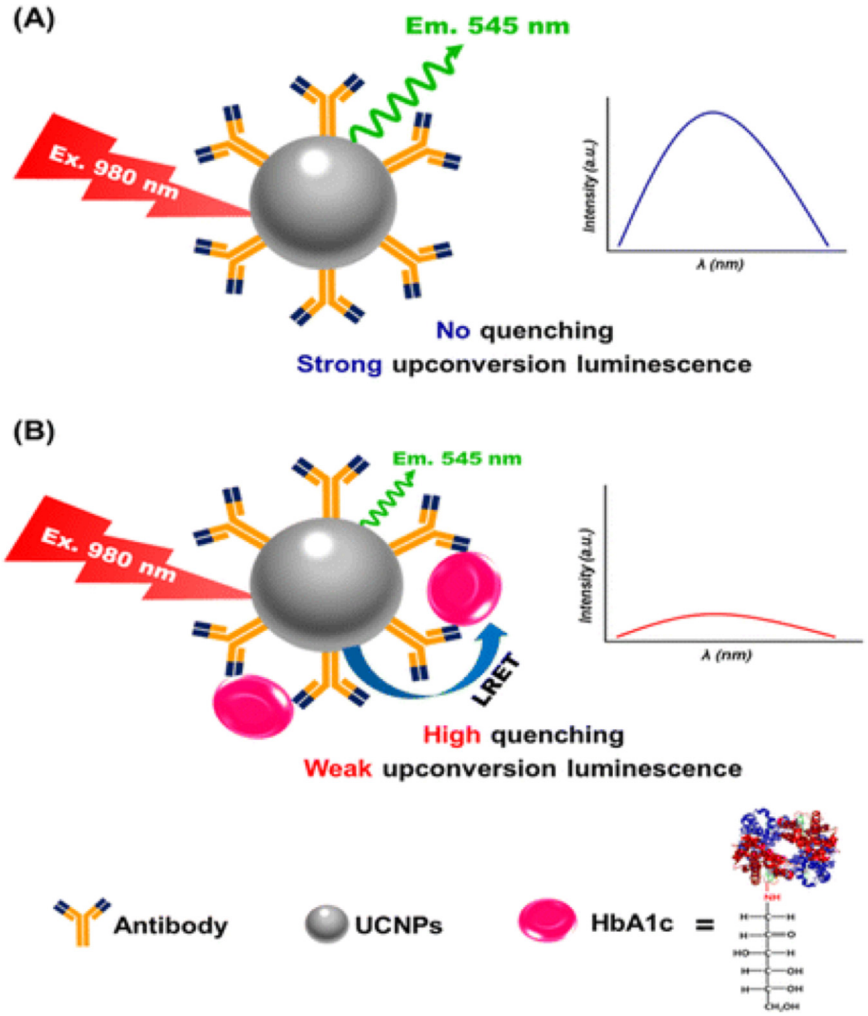

Fig. 5. LRET-based Biosensor for HbA1c Detection: (A) High luminescence, no quenching; (B) Binding of HbA1c, low luminescence, high quenching. Reprinted with permission (Jo et al., 2016).

et al., 2015). Aptamers are oligonucleotides, such as ribonucleic acid (RNA) and single-strand deoxyribonucleic acid (ssDNA) or peptide molecules that can bind to their targets with high affinity and specificity due to their specific three-dimensional structures (Ilgu and Nilsen-Hamilton, 2016) For sensing applications, aptamers, as the bioreecognition reagents, not only have all the advantages of antibodies, but also have unique merits, such as thermal stability, low cost, and unlimited applications (Shigdar et al., 2013). Recently an integrated microfluidic system based on aptamer–antibody assay on magnetic beads was developed for measurement of glycated hemoglobin levels with high specificity and sensitivity (Chang et al., 2015). This microfluidic system is low cost technique as one of the antibodies used in sandwich immunoassay is replaced with an aptamer and can also be used as POC device. The response time of developed sensor was 25 min and it required very less amount of sample (1 μL of blood). The same group developed an automated aptamer-based microfluidic system by replacing both capture and detection antibodies in a sandwich assay with specific, stable and low cost aptamers (Li et al., 2016). Use of aptamers reduced the cost of biosensor whereas increased the reliability and sensitivity which were main problems with use of antibodies. The microfluidic chip (4.3 × 5.3 cm) had a number of open chambers for loading the sample, out of which four chambers were specific for HbA1c through which HbA1c could be detected fluorescently (Fig. 6). It has been successfully applied for detection of Hb and HbA1c secreted from red blood cells. Compared to other methods used for HbA1c detection these biosensors used nearly 75% less sample volume i.e. 1 μL of blood and reaction time was reduced to 30 min from 3.5 h. The detection range of developed biosensor was 0.7–2.1 g dL^{-1} HbA1c and 10.8–14.8 g dL^{-1} Hb. With the advantages of fast detection, small sample volume, and high throughput, he microfluidic based chip device will continue to demonstrate its advantages in HbA1c analysis of point-of-care.

3.2.3. Colorimetric biosensors for HbA1c

Due to the biological meaning of HbA1c in point-of-care (POC) diagnostics, focus has been shifting to develop methods that can measure HbA1c levels directly and do not require large sophisticated instruments (Tanaka et al., 2017). A dual immunosensor for the detection of Hb and HbA1c simultaneously was developed by Ang et al. (2016b, 2015). It performs the detection of HbA1c in a single step due to its specific dual-detection design and moreover it does not require pretreatment of blood samples. This immunosensor was particularly helpful in detecting HbA1c levels in patients suffering from sickle cell anaemia, as in such patients if absolute 100% HbA1c levels are detected the results are false due to low levels of Hb. The immunosensor was developed using conjugate pad, laminated nylon membrane, application pad and absorption pad. The five test lines were used: control line (secondary polyclonal rabbit anti-goat antibody), Hb line (polyclonal anti-Hb antibody) and three HbA1c test lines (monoclonal anti-HbA1c antibody) (Fig. 7). Sandwich immunoassay was performed using antibody coated AuNPs and the signals could be observed on strip with naked eye. The detection range of immunosensor was 4.0–7.0% HbA1c

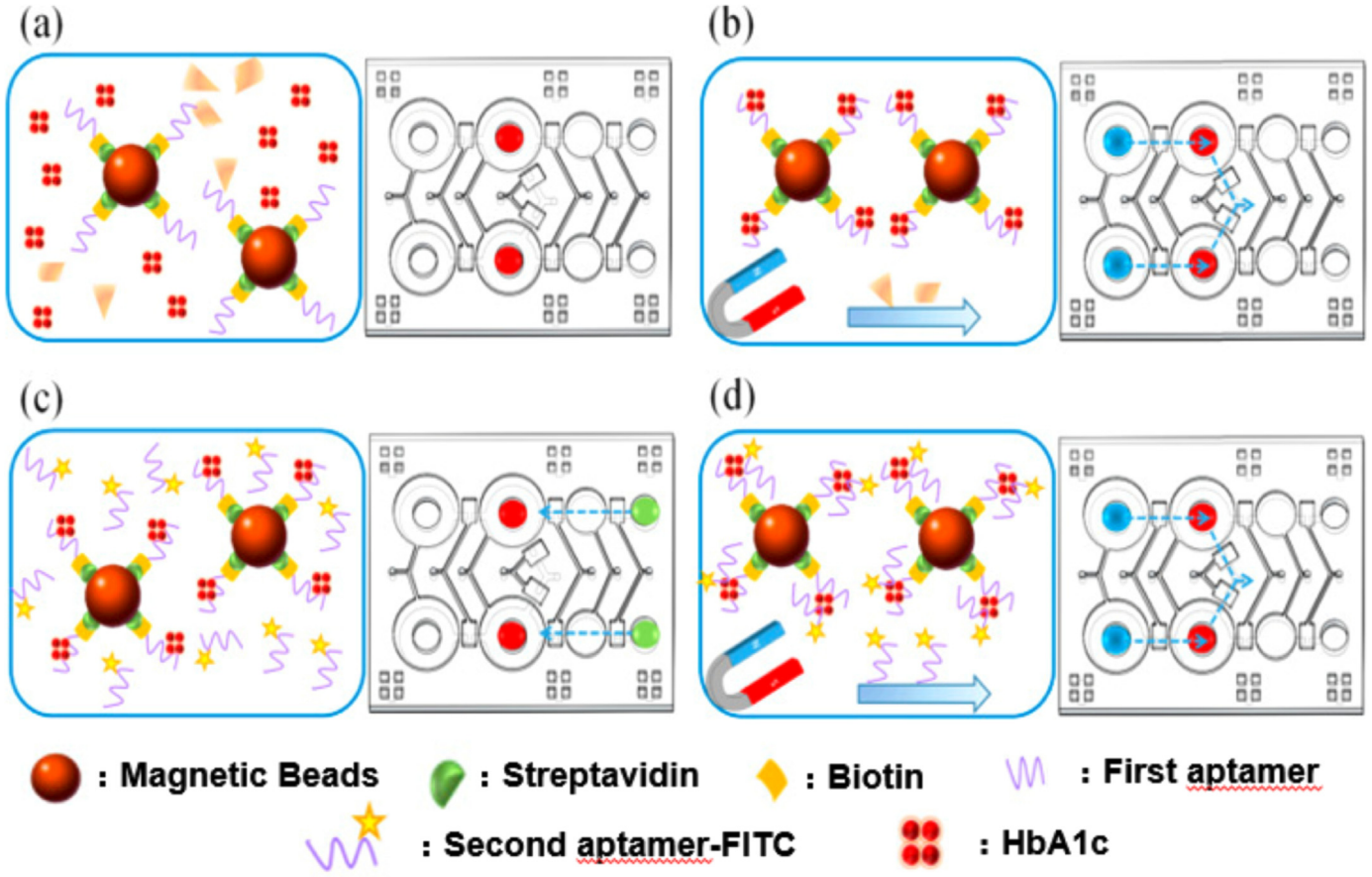

Fig. 6. Microfluidic biosensors for HbA1c detection (a) Magnetic beads coated with both aptamers and blood samples placed in closed chamber, (b) Application of external magnetic fields to collect magnetic beads and washing with phosphate buffer (0.01 M), (c) Transportation of FITC-labeled aptamers to transportation unit, (d) Sandwich reaction of blood samples-magnetic beads-aptamers and detection of fluorescent signals. Reprinted with permission (Li et al., 2016).

with response time of 20 min. The developed biosensor had advantage of being specific, sensitive and it requires only 2 μL of blood sample (Ang et al., 2016a).

Another immunosensor based on lateral flow was developed which involved the direct detection of HbA1c. Colloidal gold functionalized antibodies were used to produce specific signals on sandwich reaction on strip. The detection range of developed immunosensor was 20–130 mmol mol^{-1} or 4–14% HbA1c and detection limit was 1.35 mmol mol^{-1} or 42.5 g mL^{-1}. The immunosensor was rapid, accurate, sensitive and stable for 1.4 months. Moreover, the immunosensor does not require professional personnel to perform the test and can avoid number of clinical visits. The sensor can be commercialized as it can perform both semi-quantitative and quantitative measurements by providing test strips along with the scale to measure the intensity of lines (Ang et al., 2016b). When whole blood sample (10 μL) was added to test line 1, N-terminal valine of HbA1c binds with monoclonal antiHbA1c antibody and excess of HbA1c moves to subsequent test lines. Red lines are formed on formation of sandwich of HbA1c and anti-Hb gold conjugates. In the previous sandwich immunoassay there was steric hindrance due to immunocomplexes between immobilized anti-hemoglobin antibody and Hb or HbA1c. Moreover, the later immunosensor was able to give quantitative results (obtained by ESE-Quant lateral flow reader) compatible to those obtained clinically.

4. Standardization of HbA1c analysis

Comparing HbA1c results obtained by different laboratories is challenging since variable methods are available for detection of HbA1c levels (Little et al., 1992). Moreover, in this heavily migrating population it is very difficult for people to carry all their native records with them which cause chaos to health providers. Therefore, to ensure correlation between the results reported by different laboratories, standardization of units and methods of measuring HbA1c levels has become the top concern. Taking into consideration the need of standardization, International Federation of Clinical Chemistry (IFCC) in 1995, developed a universal calibration method for HbA1c detection. They prepared a mixture of HbAo and pure HbA1c and analyzed them using two reference assays (capillary electrophoresis or mass spectroscopy with reverse phase HPLC). From the outcomes of these assays they defined HbA1c as hemoglobin that is irreversibly glycated at one or both N-terminal valines of β-chains (Kobold et al., 1997) or any lysine residue in the β-chain. The results obtained using IFCC definition are very specific and do not include any non-HbA1c components. Questions were raised on the standardization given by IFCC as the results obtained by this definition were 1.5–2.0% lower than those given by Mono-S (Sweden), JSCC/JDS (Japanese Society of Clinical Chemistry/Japanese Diabetes Society) and National Glycohemoglobin Standardization Program (NGSP) of US (Hanas, 2002). This problem was solved by “master equation” which could give the relationship between all the results (Hoelzel et al., 2004). In 2004, five groups (IFCC, NGSP, International Diabetes Federation (IDF), European Association for the Study of Diabetes (EASD) and ADA) worked together to harmonize the testing protocol, and in 2007 it was announced that HbA1c tests results shall be articulated as mmol HbA1c/mol Hb. But this gave birth to communication gap between the physicians and patients who monitored HbA1c levels at home. These questions were answered in May 2007 by the acceptance of universal accord statement that HbA1c results must be reported in mmol glycated Hb/mol total Hb (IFCC units)

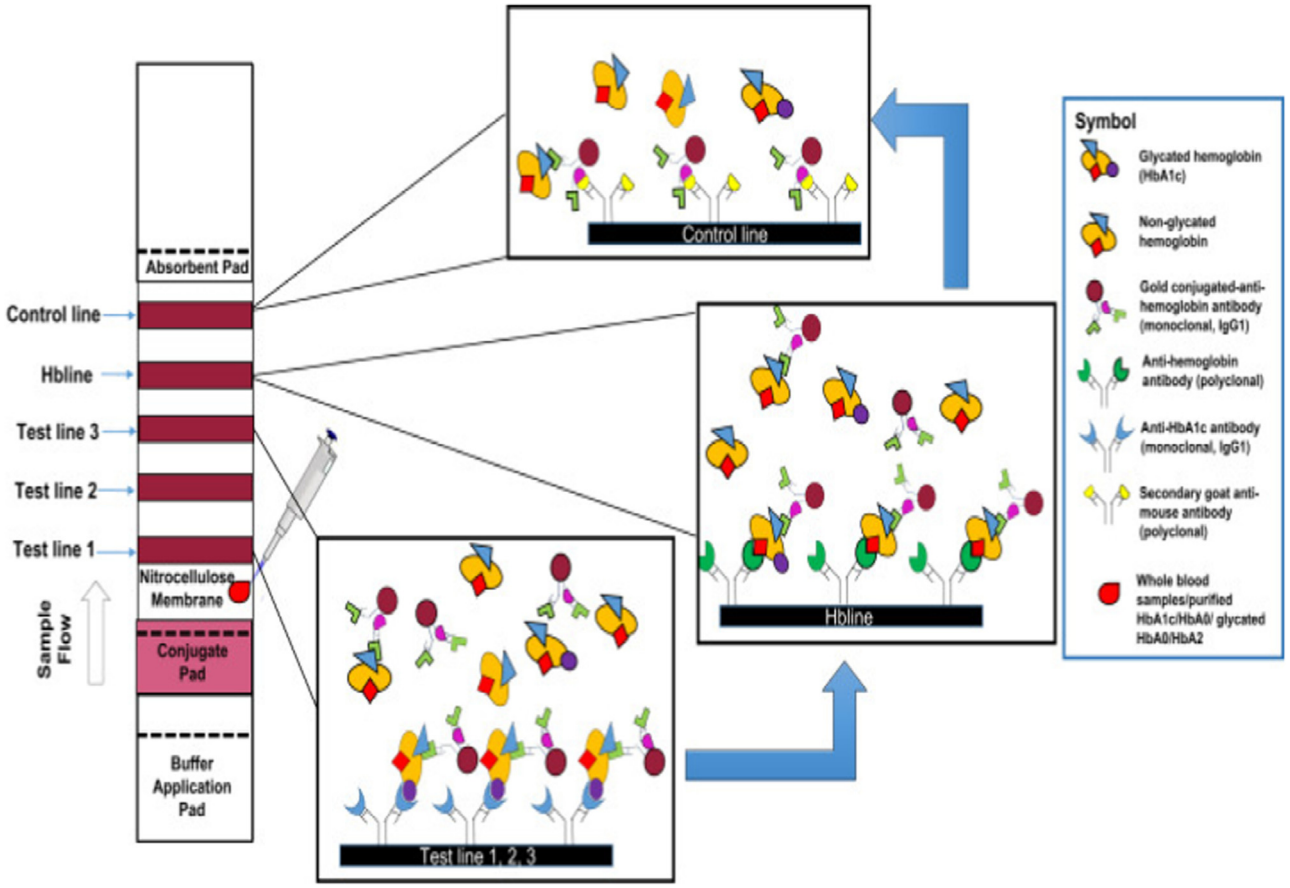

Fig. 7. Sandwich immunoassay for HbA1c detection. Reprinted with permission (Ang et al., 2016a).

Table 2
Conversion factors for HbA1c values using IFCC-NGSP master equation (Hoelzel et al., 2004).

National DCM	From IFCC to DCM	From DCM to IFCC
NGSP (USA)	0.09148 IFCC + 2.152	10.93 NGSP – 23.50
JSCC/JDS (Japan)	0.09274 IFCC + 1.724	10.78 JDS − 18.59
Mono-S (Sweden)	0.09890 IFCC + 0.884	10.11 Mono-S − 8.94

and % (derived NGSP units, obtained from national designated comparison method, DCM using IFCC-NGSP master equation) (Weykamp, 2013) (Table 2). The HbA1c values for non-diabetic patients were standardized to 25–42 mmol mol^{-1} or 2.5–4.2% (Weykamp, 2013).

The results obtained using above protocol seemed to be reliable in the beginning but later few loop hopes were found such as limited number of patients studied, fewer methods applied and no sample collection during the night time which did not represent true value of HbA1c. Therefore, continuous glucose monitoring was carried out in which the samples were taken every five min for 3 months. But the results obtained were also not reliable as patients suffering from type 1 diabetes mellitus which could not be applied to conversion equation (Nathan et al., 2007). Then, a new study was conducted in 10 different locations in Europe, Africa and North America involving 507 patients (80 non-diabetic, 268 type 1 diabetes mellitus and 159 type 2 diabetes mellitus patients) and nearly 2700 samples were collected from each patient over a period of 3 months. It was reported to be the largest number of blood samples from patients to be studied. The main aim was to report the results in average values rather than percent values. Thus, a new term, estimated average glucose (eAG) was used to express the HbA1c results instead of % HbA1c (Nathan et al., 2007). Studies have shown the advantages and feasibility of standardizing HbA1c assays (Little et al., 1992) and a reference method was proposed almost a decade ago. On an annual basis, manufacturers of both traditional and rapid HbA1c test assay methods are awarded a “certificate of traceability to DCCT reference method” if their assay method passes rigorous testing criteria for precision and accuracy. Manufacturers are awarded Certificate of Traceability if the total imprecision (coefficient of variation) is ≤ 4% and 95% CI of the difference between methods falls within ± 1%. Each certification is effective for one year from the date of certification. They must calibrate the results obtained using HPLC based on ion-exchange columns. Certified assays are listed on the NGSP website (http://www.ngsp.prg).

5. HbA1c POC analyzers in the market

As discussed earlier the clinical tools detect glucose levels only at the time of test. Therefore, HbA1c level has become the standard measure of diabetes control and lower HbA1c levels have been shown to translate into improved clinical outcomes in diabetes patients. It is essential to have POC diagnostics of HbA1c being performed in high quality clinical labs and then standardized to National Glycohemoglobin Standardization Program (NGSP) (Little, 2003). Main benefits of POC testing are that the test could be performed anywhere without need of assess to lab and the results are obtained immediately without any time lag between test time and time of visit to the physician. However, it is important that care must be taken while storing the reagents required for POC testing and only skilled professionals should perform the test. Despite these considerations the results obtained by POC are effective and quite reliable (Cagliero et al., 1999; Miller et al., 2003). Commercially available methods to measure HbA1c can be divided into laboratory instrument methods and POC methods. The two most common principles include those based on charge differences (ion-

exchange chromatography and electrophoresis), and structural differences (boronate affinity chromatography and immunoassay) for measuring glycosylated hemoglobin. Analysis of clinical laboratory proficiency surveys for glycosylated hemoglobin measurement, conducted by the College of American Pathologists in 2007, revealed that immunoturbidometric assays account for the majority (65%) of the methods used to measure HbA1c. Ion-exchange chromatography method accounted for a little more than 32% of the methods for the measurement of HbA1c, and boronate affinity accounted for a little more than 2% of the methods used to determine glycosylated hemoglobin values.

The analytical performance of laboratory instrument methods is typically better than the performance of POC methods in terms of robust, but the POC methods have the advantage of producing results during the patient's visit (Weykamp, 2013). Studies have confirmed that immediate feedback of HbA1c results improves glycemic control in type 1 and insulin-treated type 2 diabetic patients (Lenters-Westra and Slingerland, 2010). The development of POC testing methods is a recent trend and it is under consideration by the ADA for use in the screening and diagnosis of diabetes. POC methods are small instruments, or unitized devices, that run single samples. It is essential to have POC diagnostics of HbA1c being performed in high quality clinical labs and then standardized to National Glycohemoglobin Standardization Program (NGSP) (Little, 2003). However, it is important that care must be taken while storing the reagents required for POC testing and only skilled professionals should perform the test. Despite these considerations the results obtained by POC are effective and quite reliable (Cagliero et al., 1999; Miller et al., 2003). Different POC analyzers available in market are listed below in Table 3. Some popular POC HbA1c analyzers available in the market will be discussed in the following section.

5.1. POC analyzers based on cation exchange chromatography

5.1.1. DiaSTAT HbA1c analyzer (Bio-Rad Diagnostics Group)

The DiaSTAT Analyzer is the principle competitor for the DCA 2000. This bench-top instrument (20 kg) uses cation-exchange chromatography in conjunction with gradient elution to separate hemoglobin subtypes (including HbA1c) from hemolyzed whole blood; the technology exploits the differing pI values of HbA1c and HbA10 (7.4 and 6.95, respectively). The separated hemoglobin fractions are monitored by light absorption. Two other variants are the “Variant” (Bio-Rad) and “HA8140” (Menarini) represent automated HPLC chromatograph techniques.

5.1.2. DS5 HbA1c analyzer (Drew Scientific Group)

The Drew DS5 instrument, dispenser and associated reagent kit are intended for the measurement of HbA1c in blood samples. The system's small size and ease of use makes it ideal for main laboratory, clinic or satellite laboratory settings. The DS5 uses cation exchange chromatography in conjunction with gradient elution to separate human hemoglobin subtypes and variants from hemolyzed whole blood. The separated hemoglobin fractions are monitored by means of absorption of light and the chromatogram obtained is recorded and stored by the internal computer. A software program in the instrument performs the analysis of the chromatogram and generates a result report on the integral thermal printer. HbA1c result is obtained in 5 min, and the presence of HbS or HbC can be detected.

5.1.3. G7 HbA1c analyzer (Tosoh Bioscience Inc.)

The G7 is a fully automated, high performance liquid chromatography system designed to measure glycosylated hemoglobin accurately and rapidly. Tosoh's new G8 analyzer, which is nearing FDA approval, will have an elution time of 1.6 min and will use cation exchange protein separation technology. It will improve upon the current G7 version both in elution time (the G7 is 2.2 min), and by having two

Table 3
Different POC analyzers for HbA1c detection available in market.

Principle	Analyzers	Manufacturers	Response time	Sample volume	Detection range	Advantages
Cation-exchange chromatography	DiaSTAT	Bio-Rad Diagnostics Group	10 min	10 µL	2.8–15.9%	Easy to use, automated
	DS5	Drew Scientific Group	5 min	20 µL	5–13.2%	Small in size, easy to use
	G7 HbA1c	Tosoh Bioscience Inc.	2.2 min	1 mL	4.6%	Fully automated, accurate, rapid
Immunoassay and immunoturbidometry	Direct Enzymatic HbA1c Assay	Diazyme Laboratories	2 min	25 µL	5.7–10.3%	Specific, cost effective, no interference of Hb variants
	DCA 2000(+)/DCA Vantage	Siemens Medical Diagnostics	6 min	1 mL	2.5–14.0%	Accessible, accurate and easy to use
	A1c At-Home Test	Becton Dickinson	5 min	1–2 drops	2–9.4%	Convenient to both patient and health care provider
	Innovastar	DiaSys	6.5 min	10 µL	4–15%	Easy to use, precise
Boronate Affinity Chromatography	Vision Analyzer	Abbott Diagnostics	3 min	50 µL	0.9–4.7%	Stable results, unskilled operator
	NycoCard	Axis-Shield Group	3 min	5 µL	4–15%	Accurate, reliable, no interference of Hb variants
	HemaQuant Analyzer with Glycosal catridges	Provalis Diagnostics	4 min	10 µL	3–18%	Rapid
	Clover	Infopia	5 min	7 µL	4–14%	Portable, home care
Spectroscopy	A1c Now	Metrika	8 min	10 µL	0–3.34%	Easy to use, affordable, accessible

levels of flagging. The upgrade in sophistication will also include pop-up reminder windows.

5.1.4. Diazyme's direct enzymatic HbA1c assay (Diazyme Laboratories)

Diazyme's Direct Enzymatic HbA1c Assay has all the advantages of both the HPLC and immunoassays methods in the areas of accuracy, specificity, applicability to chemistry analyzers. It is also cost effective, simpler and has no interference from hemoglobin variants. The assay principle is that whole blood samples are lysed with a lysing buffer and the released hemoglobin molecules are digested with a protease mix. This process releases amino acids including glycosylated valines from the Hb β chains. Glycosylated valines then serve as substrates for specific recombinant fructosyl valine oxidase (FVO) enzyme which specifically cleaves N-terminal valines and produces hydrogen peroxide in the presence of selective agents. This, in turn, is measured using a horseradish peroxidase catalyzed reaction and a suitable chromagen. The HbA1c concentration is expressed directly as % HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in % HbA1c. This assay only needs a single channel to perform the test on chemistry analyzers in comparison with some immunoassays that require a separated measurement of total Hb and need two channels for the test on chemistry analyzers. Diazyme has received USA Food and Drug Administration (FDA) and NGSP certification for its direct enzymatic HbA1c assay product.

5.2. POC analyzers based on immunoassays and immunoturbidometry

5.2.1. CA 2000 (+) or DCA vantage (Siemens Medical Diagnostics)

The DCA 2000 (+) analyzer is the outdated version while DCA Vantage is the upgraded one manufactured by Siemens Medical Diagnostics. Both analyzers are based on monoclonal antibody method for HbA1c and some high-volume labs still use DCA 2000 (+) analyzer It is NGSP certified method providing outstanding accuracy and precise, as recommended by ADA. It requires only 1 mL of capillary/venous whole blood, or 40 mL of urine for a test and obtains HbA1c results in just 6 min. The DCA 2000 (+) is accessible, accurate, and easy to use, but it is limited to high-volume clinics because of instrument costs. DCA 2000 (+) analyzer showed 3.4–7.3% CV in a study conducted on 80 diabetic samples (Yeo et al., 2009). The analyzer could detect 2.5–14.0% or 130 mmol mol^{-1} HbA1c in venous or capillary blood. DCA Vantage is a light weight instrument weighing 3.9 kg. Different studies compared HbA1c studies with DCA 2000 (+) and DCA Vantage to find that latter gives better results (Lenters-Westra and Slingerland, 2009, 2014; Petersen et al., 2010; Sanchez Mora et al., 2011; Szymezak et al., 2008; Wiwanitkit, 2012; Zhou et al., 2014b), is user friendly and has better ergonomics (Szymezak et al., 2008) and the performance was dependent on lot number (Lenters-Westra and Slingerland, 2009).

5.2.2. B-D A1c at-home-test (Becton Dickinson)

The B-D A1c At-Home-Test combines a filter paper technique for spotting capillary blood with an immunoturbidometric assay (a variation of the Cobas Integra Hemoglobin A1c method also referred to as Roche Unimate). Patients place 1–2 drops of fingerprick blood on each of two target areas on filter paper in the test kit. The sample is left to air-dry overnight at room temperature, placed in a pre-addressed bag, and mailed to a central laboratory. Laboratory personnel elute the blood from one spot on the filter paper and perform the assay for HbA1c. The second spot is stored at − 70 °C for contingency purposes. This product may add to the convenience of both patient and health care providers by making it easier for patients to obtain their HbA1c values and by having them available at their visit to the clinician.

5.2.3. Innovastar (DiaSys)

HbA1c Direct measurement is based on particle-enhanced immunoturbidometry. The measuring range is 4–15% HbA1c. It takes 6.5 min to get the HbA1c reading, and a 10 μL capillary blood is needed for test. The device is limited to POC testing as skilled professionals are needed to operate it (Jensen, 2014). The studies conducted to check its performance showed that the instrument has bias of − 0.47 to − 0.16% (Lenters-Westra and Slingerland, 2009, 2014) and the results obtained are not reliable (Jensen, 2014).

5.3. POC analyzers based on boronate affinity chromatography

5.3.1. Vision analyzer (Abbott Diagnostics)

Vision Analyzer (Abbott Diagnostics) assays HbA1c by way of affinity chromatography. The Afinion™ AS100 Analyzer measures HbA1c in 3 min. The affinity binding of glycohemoglobin to 3-aminophenylboronic acid immobilized on a finely divided agarose support is measured via bichromatic absorbance at 553/628 nm. Abbott claims that although this affinity binding method detects glycohemoglobin species other than HbA1c, it correlates highly with more selective methods such as ion-exchange chromatography. The company also proposed a non-separation assay for glycohemoglobin, although it has not yet formed the basis of a commercial product. The assay records the fluorescence of a fluorescein-3-aminophenylboronic acid conjugate when incubated with hemolyzed blood. The fluorescence signal is reduced (quenched) upon binding to HbA1c and therefore can be used as a measure of its concentration. The result must be corrected for variations in total hemoglobin concentration.

5.3.2. NycoCard® HbA1c analyzer (Axis-Shield Group)

The NycoCard HbA1c test is a 3 min POC assay for measurement of glycosylated hemoglobin. NycoCard HbA1c provides an accurate and reliable method to monitor metabolic control in people with diabetes. It uses a boronate affinity test principle and provides high quality results without interference of Hb variants and derivatives. The test only requires a 5 μL of blood.

5.3.3. HemaQuant analyzer with glycosal cartridges (Provalis Diagnostics)

Glycosal is a rapid, POC test using the established method of affinity chromatography for the quantitative determination of HbA1c. Glycosal has a linear working range of at least 3–18% HbA1c, requires approximately 10 μL of whole blood and generates a result in approximately 4 min The Glycosal test in the cartridge uses boronate affinity chromatography to separate the glycosylated hemoglobin fraction from the non-glycosylated fraction. The blood is lysed to release the hemoglobin and the boronate affinity resin binds the glycosylated hemoglobin. After a short incubation step, the unbound non-glycosylated hemoglobin is measured photometrically, then the boronate affinity resin is washed and the bound glycosylated hemoglobin is eluted from the resin and measured; the HbA1c concentration is calculated by the instrument.

5.3.4. Clover (Infopia)

The Clover HbA1c analyzer is based on boronate affinity binding precipitation/electrochemical reaction. The measuring method is spectrophotometry/amperometry. The test range is 4–14% HbA1c, and it is a 5 min assay. A 7 μL sample of whole blood is needed to test. It is a portable/home care system and can measure HbA1c economically.

5.4. POC analyzers based on spectroscopy

5.4.1. A1cNow (Metrika)

The A1cNow test is a single use, disposable, battery powered device. The device uses Micro-Optical Detection Method (MODM™) technology that incorporates microelectronics, optics, and dry-reagent chemistry strips within a cassette sized plastic casing. It contains two dry reagent lateral flow strips, each having an HbA1c immunoassay test zone and a total hemoglobin test zone. With the addition of the sample, blue microparticles conjugated to anti-HbA1c antibodies (sheep polyclonal antibodies) migrate along the reagent strips. The amount of blue

microparticles captured on the immunoassay zone of each strip reflects the amount of HbA1c in the sample. For the total hemoglobin portion of the assay, the sample diluents, which contains ferricyanide, converts hemoglobin to met-hemoglobin, which is red-brown in color. The intensity of the color measured on the second test zone of each reagent strip is proportional to the concentration of hemoglobin in the sample. Assay results are then calculated from the reflectance of the test zones by an onboard microprocessor and are expressed as %HbA1c. A 10 μL sample of whole blood is needed to test, and test results (%HbA1c) are displayed as a number on the device's display within 8 min after sample application. A1CNow+ is accessible, accurate, and easy to use while remaining affordable to both primary and specialist practices.

6. Summary and conclusions

HbA1c level has become the standard measure of diabetes control. A number of strategies such as chromatography, biosensors and POC methods have been developed for detection of HbA1c levels in patients. Chromatographic techniques suffer from disadvantages like need of skilled operator and long assay time. Therefore, various biosensors were fabricated for HbA1c detection. All the biosensors are reported to meet the clinical demand for measuring the HbA1c/total hemoglobin ratio of 5–20%. However, the performance of these biosensors is compromised by the interference from the HbA1c variants due to the application of ferroceneboronic acid on the sensing interface and HbA1c variants also have affinity for the latter (Rhea and Molinaro, 2014). Designing new boronate probes which have high affinity will be future solution to increase the specificity. Moreover, the stability of sensing interface is affected by the sensitivity of interface to light and air, and the poor long-time stability of redox probe ferrocenedimethylamine which is frequently used in electrochemical biosensors. We have demonstrated that aryldiazonium salt chemistry is helpful to form stable interface chemistry with rich chemistry (Cao et al., 2017). Due to the clinical importance of HbA1c, POC methods will be the end-user friendly approaches for monitoring circulating HbA1c in blood. But, the optimal use of HbA1c results requires standardization of these POC test assays to ensure reported results between laboratories are comparable. All the different POC HbA1c tests are similar with regards to accuracy, time to result, etc. However, success of the different products is largely dependent on the sales and distribution capabilities of manufacturers.

7. Future perspectives for HbA1c detection and diabetes management

Although a number of reliable techniques are available for the detection of HbA1c still for diagnosis and management of diabetes, there are few gaps that need to be covered. Development of immunosensors are still the popular strategy to develop HbA1c sensors. In addition to consider the stability, special considerations for sensing interface design with both components of anti-biofouling and bio-recognition needs to be taken into account allowing sensors reliably working in whole blood samples (Jiang et al., 2016, 2017). Additionally, platforms that offer expanded testing capabilities are looked upon favorably and may be adopted more quickly than single analyte systems. Besides immunosensors, the use of aptamer-based biosensors is a new trend for developing assays for HbA1c due to the stability and low cost of aptamers. It will provide POC testing of HbA1C in a cost-effective way and make it affordable to a wide population of patients. As the diabetes epidemic continues to spiral, there will be many opportunities for companies to strive for advances in detection and treatment, and they will surely outdo one another in technological achievements in both POC and clinical lab applications. All new HbA1c test methods should meet the technology assessment criteria: 1) The technology must meet final approval from the appropriate government regulatory bodies. 2) The scientific evidence must permit conclusions concerning the effectiveness of technology regarding health outcomes. 3) The technology must provide net health outcomes. 4) The technology must be an improvement on any established alternatives. 5) The improvement must be attainable outside investigational settings. Guaranteed result quality is mandatory and must not be sacrificed for patient or clinician convenience. Also, it is expected that all the analyzers will use one standardized unit to represent HbA1c concentrations. This will simplify the interpretation between the patients and clinicians which may further help in better diabetes measurement and management.

With the aid of robust surface chemistry, nanotechnology and advanced analytical sciences (e.g. soft electronics, pixel array platform, machine learning), we will expect more convenient, accurate, non-invasive and reliable assays for simple and quick quantification of HbA1c for diabetes diagnosis and control (Ali et al., 2015; Lee et al., 2017; Lipani et al., 2018). Deep machine learning algorithms and convenient app on mobile phone can be used to analyse, predict and manage the diabetes (Kavakiotis et al., 2017; Mhaskar et al., 2017), which is helpful to overcome the current drawbacks with diabetes measurement such as (1) restriction on one type of diabetes; (2) lack of understandability and explanatory power of the techniques and decision; (3) failure of prediction purpose or management over the structured contents; and (4) weakness on competence for dimensionality and vagueness of patient's data. This new wave of analytics and technology could dramatically help cut costly and unnecessary hospitalizations while improving outcomes for patients. We are expecting to get accurate and quick diagnostic report within seconds to minutes time scale.

Acknowledgment

This work was financially supported by funding from the ARC Future Fellowship (FT160100039), the ARC Centre of Excellence for Nanoscale BioPhotonics CE140100003, and the National Natural Science Foundation of China (Grant 21575045). Jagjit Kaur would like to thank the University of New South Wales for providing Ph.D. Scholarship.

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