Bioanalytical Reviews

Frank-Michael Matysik *Editor*

Trends in Bioelectroanalysis

6
Bioanalytical Reviews

Aims and Scope

Bioanalytical Reviews is the successor of the former review journal with the same name, and it will complement Springer's successful and reputed review book series program in the flourishing and exciting area of the Bioanalytical Sciences.

Bioanalytical Reviews (BAR) publishes reviews covering all aspects of bioanalytical sciences. It therefore is a unique source of quick and authoritative information for anybody using bioanalytical methods in areas such as medicine, biology, biochemistry, genetics, pharmacology, biotechnology, and the like.

Reviews of methods include all modern tools applied, including mass spectrometry, HPLC (in its various forms), capillary electrophoresis, biosensors, bioelectroanalysis, fluorescence, IR/Raman, and other optical spectroscopies, NMR radiometry, and methods related to bioimaging. In particular the series volumes provide reviews on perspective new instrumental approaches as they apply to bioanalysis, and on the use of micro-/nano-materials such as micro- and nanoparticles. Articles on μ-total analytical systems (μ-TAS) and on labs-on-a-chip also fall into this category.

In terms of applications, reviews on novel bioanalytical methods based on the use of enzymes, DNAzymes, antibodies, cell slices, to mention the more typical ones, are highly welcome. Articles on subjects related to the areas including genomics, proteomics, metabolomics, high-throughput screening, but also bioinformatics and statistics as they relate to bioanalytical methods are of course also welcome. Reviews cover both fundamental aspects and practical applications.

Reviews published in BAR are (a) of wider scope and authoratively written (rather than a record of the research of single authors), (b) critical, but balanced and unbiased; (c) timely, with the latest references. BAR does not publish (a) reviews describing established methods of bioanalysis; (b) reviews that lack wider scope, (c) reviews of mainly theoretical nature.

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Frank-Michael Matysik
Editor

Trends in Bioelectroanalysis

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Preface

Since the fundamental studies by Galvani and Ritter in the late eighteenth century, it has been understood that there is a close relationship between electrical and biological phenomena. Currently, bioelectrochemistry is a very active field, with extensive research devoted to studies of biopolymers (DNA, proteins), bioenergetics, biomembranes, and electrochemical applications in medicine or biotechnology. Bioelectroanalysis represents a vital branch of bioanalytical chemistry. According to its interdisciplinary character, electroanalysis integrates aspects of bioelectrochemistry and analytical chemistry. Bioelectroanalytical studies comprise either the incorporation of biological systems and principles into an electrochemical concept or the use of electrochemical methods to generate analytical information regarding biomolecules/biological compartments.

In this third volume of the series *Bioanalytical Reviews*, a selection of trendsetting topics in the field of bioelectroanalysis is presented. Ocvirk and colleagues contribute a comprehensive overview concerning the electrochemical biosensing of glucose for diabetes care. Due to enormous practical importance, this research area has attracted continuous interest for decades. The authors provide a detailed review from an industrial research and development perspective. Cortón and Mikkelsen present a survey of bioassay applications for individually addressable electrochemical arrays, illustrating the significant progress in this field. The focus is on liquid-phase bioanalytical assays. The context of implementation of electrochemical arrays is continued by del Valle who discusses recent advances in the development of electronic tongues based on the use of biosensor arrays coupled with advanced chemometric data analysis. Vyskocil and Hájková summarize novel strategies of DNA biosensor development and corresponding applications for studies of DNA damage. The contribution by Vatsyayan represents the important research area of electrochemistry of redox proteins. Recent trends, including increasing diversity of redox proteins used in electrochemical studies, novel immobilization strategies, and biosensor/biofuel cell applications, are surveyed. The review by van der Weerd et al. provides an overview concerning ongoing developments in the field of

electrochemical sensing of blood gases with advanced sensor concepts. Last, but not least, L. Nagy and G. Nagy address the important aspects of bioelectroanalytical studies with high spatial resolution. Their contribution summarizes recent achievements in bioanalytical applications of scanning electrochemical microscopy. A wide range of applications covering imaging of living cells, studies of metabolic activity, imaging of local enzyme activity, and studies of transport through biolayers are surveyed.

I wish to express my appreciation to all of the authors who contributed to this book project with in-depth review articles illustrating current trends in the research area of bioelectroanalysis. This timely collection of important activities in bioelectroanalytical research directions will be of interest not only for experts in the field but also to students and their teachers in disciplines that include analytical chemistry, biology, electrochemistry, and various interdisciplinary research areas.

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Contents

Electrochemical Glucose Biosensors for Diabetes Care 1
Gregor Ocvirk, Harvey Buck, and Stacy Hunt DuVall

Electrochemical Arrays for Bioassay Applications 103
Eduardo Cortón and Susan R. Mikkelsen

Bioelectronic Tongues Employing Electrochemical Biosensors 143
Manel del Valle

Novel Electrochemical DNA Biosensors as Tools for Investigation and Detection of DNA Damage 203
Vlastimil Vyskočil and Andrea Hájková

Recent Advances in the Study of Electrochemistry of Redox Proteins . . . 223
Preety Vatsyayan

Trends in Electrochemical Sensing of Blood Gases 263
Bastiaan van der Weerd, Rudolf Bierl, and Frank-Michael Matysik

Application of Scanning Electrochemical Microscopy in Bioanalytical Chemistry 281
Lívia Nagy and Géza Nagy

Index 341

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Electrochemical Glucose Biosensors for Diabetes Care

Gregor Ocvirk, Harvey Buck, and Stacy Hunt DuVall

Abstract Blood glucose monitoring (BGM) is the most successful application of electrochemical biosensor technology and has motivated tremendous improvements in biology, chemistry, measurement, and fabrication methods of biosensors. The performance of electrochemical biosensors used for BGM has improved greatly over the last four decades. Technological advance has allowed to measure blood glucose (BG) over a wide range of glucose concentration, a wide temperature and hematocrit range in the presence of an abundance of interfering substances with ever-increasing accuracy, and precision in minute sample volumes. The use of optimized enzymes, mediators, and electrochemical measurement methods enables this tremendous progress in performance. Continuous glucose monitoring (CGM) systems based on minimally invasive amperometric sensors, inserted into the subcutaneous tissue, have significantly improved over initial offerings over the last 15 years with regard to time of use, accuracy, reliability, and convenience due to a multitude of parallel advances: materials needed for enzyme immobilization, polymeric cover membranes, and biocompatible coatings needed to tackle the response by the complex body interface have been developed; wireless transfer and processing of unprecedented data volume have been established; effortless and painless insertion schemes of ever smaller sensors have been realized in order to overcome the concerns of persons with diabetes (PwDs) to use a minimally invasive sensor; and scalable manufacturing technologies of miniaturized minimally invasive sensors have allowed for ever improved reproducibility and increased production volume. Looking ahead, the demands on blood glucose system performance

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are expected to grow even as the pressures to lower the cost of systems increase. The drive for the future is to continue to push the limits on system performance under real-life conditions while lowering cost, all while finding ways to provide the best medical value to PwDs and healthcare providers. Technical issues of commercially available CGM sensors remain to be solved which currently impede reliable hypo- and hyperglycemic alarms, safe insulin dosing recommendations, or insulin pump control at any time of use. It is realistic to assume that continuous glucose monitoring (CGM) systems will be adopted in the future by a larger population of PwDs. Yet it is also clear that BGM systems will remain a major choice of the great majority of PwDs on a global scale. This review offers a technical overview about user, system, and major regulatory requirements and available suitable sensor technology and demonstrated performance of electrochemical BGM and CGM systems from an industrial R&D perspective.

Keywords Continuous glucose monitoring • Diabetes care • Electrochemical biosensor • Enzyme electrode • Medical device • Patient with diabetes • Self-monitoring of blood glucose • Sensor coating • System performance • System requirements

Contents

1 Introduction 3
2 General Aspects of Glucose Measurement 4
2.1 Requirements for Development, Manufacture, and Sale of Glucose Sensors for Diabetes Management 4
2.2 Glucose Measurement Systems for Different Use Cases 7
2.3 Glucose Measurement Technology 9
3 Self-Monitoring of Blood Glucose (SMBG) 11
3.1 Requirements 12
3.2 Monitoring Systems 15
3.3 Performance of Systems Used for SMBG 24
3.4 SMBG Outlook 37
4 Continuous Glucose Monitoring (CGM) 38
4.1 Requirements 38
4.2 Monitoring Systems 44
4.3 Performance of CGM Systems 68
4.4 CGM System Outlook 74
5 Conclusion 75
References 77

Abbreviations

BGM	Blood glucose monitoring
CGM	Continuous glucose monitoring
FAD-GDH	FAD-dependent glucose dehydrogenase
FBGC	Foreign body giant cell
GOx	Glucose oxidase

HCP	Health care provider
ISF	Interstitial fluid
MARD	Mean absolute relative difference
PARD	Precision absolute relative deviation difference
POC	Point-of-care
PQQ-GDH	Pyrroloquinoline quinone-dependent glucose dehydrogenase
PwD	Person with diabetes
SMBG	Self-monitoring of blood glucose
T1	Type 1 diabetes
T2	Type 2 diabetes
VEGF	Vascular endothelial growth factor

1 Introduction

Glucose measurement has become an essential part of medical diagnostics, particularly for persons with diabetes (PwDs) [1–3]. It is a broad topic, encompassing glucose measurement in different sample matrices, such as blood, plasma, serum, and urine, as well as different settings, for instance, so-called home use (self-monitoring of blood glucose, SMBG), continuous glucose monitoring (CGM), point-of-care blood glucose monitoring (POC), and clinical glucose analyzers.

Over the last 50 years, tremendous advances have been made in glucose biosensing from the first proposal of a glucose measuring device based on glucose oxidase (GOx) and an oxygen-sensing electrode in 1962 [4]. Glucose biosensors encompass the major portion of the biosensor market at approximately 85% of the global market [5]. The success of glucose monitoring, particularly SMBG, is remarkable for both the impact on PwD health and commercialization of the technology. There are several other markers that are useful for diabetes management, for example, HbA_{1c}, ketone bodies, fructosamine, and glycated albumin. While some of these markers are critical for long-term management of diabetes (HbA_{1c}) or immediate medical crisis (ketone bodies), none have had the most wide-reaching utility and benefit for PwDs as reliable glucose measurement [2, 6–11]. Reduction in HbA_{1c} has been shown to be closely tied to effective glycemic control in which glucose monitoring plays an integral role.

For a PwD, the following glucose measurement methodologies may be used; clinical analyzer measurements of plasma or serum levels of blood glucose are used for the diagnosis and treatment of diabetes [2, 12, 13]; POC blood glucose monitors are used for monitoring blood glucose in a hospital or clinic scenario [14, 15]; SMBG is used for monitoring of blood glucose outside of a healthcare setting [16, 17]; and CGM is used to monitor a PwD's glucose profile with high time resolution as an adjunct to SMBG. SMBG and CGM are the focus of this work.

2 General Aspects of Glucose Measurement

2.1 Requirements for Development, Manufacture, and Sale of Glucose Sensors for Diabetes Management

Glucose sensors for diabetes management are either in vitro diagnostic devices (BG) or medical devices (CGM), and as such the development, manufacture, sales, and distribution are strictly regulated by government agencies in all significant markets. In the United States, the Food and Drug Administration (FDA) regulates all aspects of the process and approves devices for sale in the United States. In the European Union (EU), CGM systems need to comply with respective medical device directives (90/385 or 93/42 EC), and the conformity assessment requires the involvement of a notified body. Devices which receive the Conformité Européenne (CE) mark may be sold in all EU countries. Several recent publications provide useful overviews of the requirement of the FDA and in the EU [18–21] and on efforts to standardize and harmonize requirements [22].

In the USA, blood glucose sensors for diabetes management are classified as class II devices, meaning they present a moderate risk of harm to the user in case of device failure. In the USA, sensors used in laboratory settings and sensors for BGM are approved through the process of a 510(k) or premarket notification. In this process, the manufacturer must provide evidence to the FDA that the product is substantially equivalent to a product already marketed, referred to as the predicate device. This initially referred to products marketed at the time of the establishment of the FDA authority over medical devices (1976). In contrast, sensors for CGM are classified as class III devices and therefore have to date been approved through the premarket approval (PMA) process. This is a more stringent evaluation, which requires a reasonable assurance, based on valid scientific evidence, that the device is safe and effective in fulfilling the intended use.

In Europe, sensor systems for in vitro glucose monitoring underlie the in vitro Diagnostic Medical Devices Directive 98/79EC(IVDD), which lists “Essential Requirements” regarding design, production, and labeling, which must be fulfilled prior to marketing the system. Sensor systems for professional use require manufacturer self-certification of conformance with the directive. Personal use systems for BGM require a conformity assessment which includes the involvement of a notified body. The medical device directives 93/42EC (MDD) establish the classification and approval framework for invasive glucose monitoring systems. Sensors for single 7-day use are classified as class IIa devices, presenting low-to-medium risk of harm to the user. CGM sensors used for longer than 7 days are classified as class IIb devices, and require testing to verify biological safety and lack of cytotoxicity. The manufacturer must demonstrate to a Notified Body that the requirements of the MDD have been satisfied. The product is then marked with a CE mark and may be sold within the European Economic Area (EEA).

The regulatory framework for medical devices begins long before the approval for marketing a product with design controls over the development process and the establishment and maintenance of a suitable Quality System according to 21 CFR

820 [23] or ISO 13485. Since single-use devices cannot be tested after manufacture and prior to use, the materials and processes used to produce the devices must be validated to ensure that the resulting product performs according to specification. For the high-volume processes used for production of BGM sensors, this is by far the most time-consuming and costly part of the product development process. Material specifications must be established and validated with analytical procedures and limits testing. Process equipment must be specified, qualified and validated, and limits of all process parameters established and tested. Packaging and labeling is also a significant part of the product and subject to the same standards.

Testing and validating the performance of the sensors requires testing on human subjects, primarily PwDs, both through the development process and to provide data for the required regulatory submissions. These evaluations fall under the regulations which govern the conduct of clinical trials. In the USA, this requires the approval and oversight of the trials by an Institutional Review Board (IRB), development of the appropriate protocols for recruitment of subjects, execution of the study, evaluation and documentation of results, and reporting of adverse events [24]. In Europe the parallel structure requires the approval and monitoring of studies by an Ethics Committee. The documentation overhead significantly increases the development time and cost.

Devices must then be manufactured according to validated processes and records created and maintained to establish that fact. The manufacturer must also maintain a system for documenting and analyzing customer inquiries and complaints. A system for Corrective and Preventive Action (CAPA) must be established and maintained. Reports of problems or product malfunctions must be investigated, and in serious cases, the product might need to be recalled from the market, at great expense. 21 CFR 806 [25] gives detailed instructions for the handling of notifications and product recalls in the USA. Deaths and serious injuries which a device has or may have caused or contributed to and certain device malfunctions must be reported to the responsible regulatory agency. In the USA, a medical device report (MDR) is required to be filed with the FDA. Records of all of these systems and processes must be complete and made available to the agency on request. 21 CFR 803 [26] details the requirements for medical device reporting in the USA. In the EEA, a manufacturer must establish and maintain a systematic procedure to review experience and report to the competent authority any malfunction, failure, or deterioration (see MEDDEV 2.12-1 for further details [27]).

Some practical and commercially important aspects of the development, production, and sale of sensors are not regulated but vital for the commercial success of a product. Production processes are typically scaled for the projected sales quantities, and in the case of BGM sensors, this can be in the billions of sensors per year. While the lower volume processes of the smaller manufacturers can be scaled through parallel replication, this is not economically efficient at the scales required by the major manufacturers. The efficiencies derived from highly automated processes allow the high-volume producers to maintain very high quality at sufficiently low unit production costs. A highly automated process requires significant capital investment in design, installation, and qualification, so the process must be

correctly scaled to the expected volume. Scaled-up production of medical device prototypes is more complex than frequently assumed [28]. Scalable manufacturing methods must be employed early on in the development cycle to ensure production of representative functional test models and prototypes and also in order to ensure that test performance which can be achieved with prototypes can be replicated on a production level. Given the expense of establishing a highly automated and validated process, thorough development of a product design, which meets all performance and handling aspects, must precede investment into particular manufacturing equipment. It was rightly pointed out [29] that an insufficient design cannot be compensated for by good manufacturing practice.

Reliable delivery of product requires suppliers which reliably deliver the materials in the required quantity and quality over product lifetime. Often, second or even third sources for critical reagents and materials must be developed and validated to ensure supply reliability. A material which is used for other purposes than medical device manufacturing can be particularly challenging, as specifications or material property changes which have no impact on most users may significantly impact the medical device manufacturer. For example, a polymeric material which is suitable for nonmedical use may not be applicable for an implanted CGM sensor due to sterilization requirements and subsequent necessity of proving toxicological safety. Materials which can be used in a consumer product may not be able to be combined in a medical device due to material interactions over time of storage which leads to deterioration of performance or even safety.

BGM sensors are rarely shipped directly from the manufacturer to the end user, but typically require a distribution chain of shippers, wholesalers, and retailers. The product must be stable at the conditions required for the time needed to reach the user, which can be up to 18 months, and the storage conditions must be monitored to verify compliance to the product limits. In the case of BGM sensors, which are typically provided in a primary package of 50 sensors, they must also be stable throughout the use time until the package is empty. The package is usually provided with a desiccant, such as silica gel, to maintain a dry environment for the reagent. This package will be opened and closed up to 50 times in the uncontrolled environment of the user. The sensors and desiccant must survive this repeated opening and closing even in challenging environments, such as high temperature and high humidity. Sensors which have been exposed to conditions which might render them inaccurate should be detected in some fashion, and not be allowed to report a value [30].

CGM sensors are currently shipped directly to the user in smaller packages, since they may have shorter expiration dating after sterilization. However, room temperature storage, equally a standard for BGM and CGM sensors, requires robust sensor designs and extensive testing prior to product launch for both applications.

2.2 Glucose Measurement Systems for Different Use Cases

Different use cases in diabetes care require different glucose measurement systems. Three types of setting for glucose measurement can be defined as clinical, healthcare provider (HCP), and patient self-monitoring, including both SMBG and CGM. Each of these settings has different requirements for performance and regulatory approval. In addition, the requirements for regulatory approval are different, depending on the market the product is sold in. The performance and regulatory requirements for SMBG and CGM are discussed in their respective sections.

Glucose measured on clinical systems, which are also called Central Laboratory Systems, are the primary method for diagnosing diabetes mellitus because these instruments are able to provide the high degree of accuracy in glucose measurement needed. These instruments are generally large and perform the glucose measurement under very controlled conditions. The majority of clinical analyzer systems are based on optical measurements. An exception is the 2300 STAT Plus Glucose and Lactate Analyzer, produced by YSI Life Sciences, which is based on an electrochemical method using immobilized GOx [31].

Since lab analyzers are based on a variety of analytical methods, requiring different pre-analytical analytical steps and measurement methodologies, the time required to obtain a glucose measurement varies widely with the type of instrument used. The measurement time to acquire the glucose measurement may range from a little over 1 to 15 min. When sample handing and pre-analytical steps are taken into account, the time from collecting the sample to obtaining a glucose result may take up to 90 min, whereas the time to complete a POC blood glucose measurement is approximately 5 min [32–34]. One consequence of the variability in test time and analytical methodology for clinical analyzers is the significant system-to-system variability, as well as laboratory-to-laboratory variability, in reported glucose values [35, 36]. The trade-off for the potential excellent system performance for clinical analyzers is longer time for a final glucose value.

POC glucose testing is defined as glucose testing which is performed at or near where the patient is located, e.g., in a hospital or in a healthcare provider setting, outside of a clinical laboratory with a portable instrument. POC testing is generally held as separate from SMBG, given the difference in requirements for testing in a POC setting, such as hygiene, system suitability checks, sample type (capillary, venous, and arterial blood), and accuracy. POC systems often have more specialized data management requirements as well, where patient information and test results are tracked in a patient data management system.

CLSI has established guidelines for the use of POC glucose monitoring systems and the controls around such systems through consensus, POCT12-A3 [37] and POCT13 [15]. In these guidelines, practices to ensure proper hygiene, quality control (including training and operator proficiency requirements), performance requirements, and documentation are described. POCT12-A3, for use in acute and chronic care facilities with laboratory support, describes acceptable performance

for POC meters when at least 95% of measured glucose values are within ±12 mg/dL (glucose values below 100 mg/dL) and 12.5% (glucose values at or above 100 mg/dL) of a reference value and when at least 98% of the measured glucose values are within ±15 mg/dL (glucose values below 75 mg/dL) and 20% (glucose values at or above 75 mg/dL). These performance requirements are more stringent than what is required for over-the-counter (OTC) SMBG systems. POCT13, for use in facilities without laboratory support, suggests that the ISO 15197 standard [17] is used for system performance and that the user be aware of limitations for use for the system in use, particularly with respect to interferences. In both guidelines, requirements for an infection control program are suggested as a means of minimizing the risk of blood-borne pathogen transmission when a single device is used with multiple patients, including wearing gloves during the testing procedure and changing gloves between patients, hand washing immediately after glove removal, using a unique lancing device which is assigned for a single patient which is never used for multiple patients, and assigning a BG meter to a particular patient unless a system can be adequately cleaned between uses for multiple patient use.

The FDA has also issued a draft guidance regarding the manufacture and use of blood glucose monitoring systems for POC use [14]. This guidance contains similar information to POCT12-A3 and POCT13, but is geared toward the manufacturer of POC blood glucose devices. The FDA guidance contains more specific and stringent requirements, especially with respect to cleaning and disinfection procedures, as well as performance requirements. Descriptions of performance testing, such as precision, intermediate precision, accuracy in the hands of the intended user with each claimed sample type (e.g., capillary, arterial, venous, or neonate), and interfering substances, are detailed in this guidance. Of particular note, the accuracy requirements are more stringent that what are laid out in ISO15197, 2013, with the requirement that 99% of values are within ±10% of the reference method for glucose values ≥70 mg/dL and ±7 mg/dL for glucose values <70 mg/dL. A further requirement is that no individual glucose measurement may be outside the specified range of ±20% of reference value for glucose values ≥70 mg/dL and ±15 mg/dL for glucose values <70 mg/dL. In addition, the draft guidance states that the system should be able to measure blood glucose accurately over the range of 10–500 mg/dL. The draft guidance has elicited much commentary from both experts and industry, both positive and negative. Some researchers and users applaud the new guidance [38–40]. While it is generally recognized that POC blood glucose monitoring systems should continuously improve performance to improve patient safety, some groups object to the performance requirements in the draft guidance as technically infeasible and will result in the loss of availability or change in device characteristics of POC systems [38, 41, 42].

While most POC blood glucose systems are based on electrochemical measurements, there is an optical system produced by HemoCue, the HemoCue® Glucose 201 system. Examples of electrochemical POC systems include Roche's Accu-Chek® Inform II and Nova Biomedical's StatStrip™.

SMBG and current CGM are "home-use" scenarios, where the PwD is the primary tester and are intended to provide the user with glucose information that

allows them to manage diabetes on a day-to-day basis. While the general testing principal for SMBG and CGM is often the same, i.e., electrochemical methods, the characteristics of the sensors and the frequency that a BG value is produced are very different. SMBG systems are in vitro systems where a single BG value is obtained from a PwD after performing a finger stick by a lancing device, which is a medical device. CGM systems are medical devices which are based on minimally invasive sensors that measure glucose in interstitial fluid with a frequency of minutes and remain implanted from 5 to 14 days. The specific characteristics of electrochemical SMBG and CGM systems are the focus of this review and are discussed in Sects. 3 and 4, respectively.

2.3 Glucose Measurement Technology

The many commercially successful glucose measurement systems available today for diagnosis and monitoring of diabetes all require sampling of a body fluid and involve the use of enzyme-based assays. Attempts to accurately measure glucose, or at least predict its concentration from its effects on the body tissue, without a blood or tissue fluid sample, have been pursued as long as continuous sensor technology. While the promise of noninvasive glucose monitoring [43, 44] remains a strong desire among PwD, the unremarkable spectroscopic characteristics of glucose and interfering physical and chemical properties have to date prevented achievement of the required performance. Nonenzymatic glucose sensors currently find application only in areas where specificity is not required, when no other reducing sugar is present, or when specificity is achieved through some previous process. Nonenzymatic glucose sensors are either based on direct oxidation of glucose on an electrode or on affinity-based glucose assays. Direct oxidation-based approaches have received increased interest due to increased nanomaterials research [45–48]. Arylboronic acid, used for affinity separation of glycoproteins, has frequently been the basis for affinity-based assays coupled with optical or electrochemical detection schemes [49, 50].

Glucose measurement of biological samples in the clinical laboratory is historically primarily based on enzyme-based spectrophotometric assays. The current standard laboratory tests use GOx/peroxidase, hexokinase and glucose-6-phosphate dehydrogenase, or glucose dehydrogenase. These assays are robust, highly specific, and economical. The recent development of assays for biological samples which can be directly traced to NIST reference material [51] has improved the prospects for standardization across different laboratory methods and sites. The first product for decentralized or individual use, the CLINITEST® (still available as the BAYER® CLINITEST®) for reducing substances in urine samples, used Benedicts' reaction employing the chemical reduction of Cu(II) to Cu(I). This test tube and reagent tablet-based assay was followed by Clinistix®, also for urine samples, but now employing the very specific enzyme GOx, along with peroxidase to generate a color change from the hydrogen peroxide produced. The reagents were impregnated in

paper pads laminated to plastic substrates to form the first generation of test strips. [52–54]. Personal diabetes monitoring was greatly advanced by colorimetric test strips for blood glucose measurement, which evolved from the urine test strips. DextroStix® (Ames/Miles/Bayer) and Chemstrip BG® (Boehringer Mannheim/Roche) began the success story of test strips with photometric readout [52] and the currently widespread practice of diabetes self-management [55].

The introduction of the enzyme electrode for medical monitoring by Clark and Lyons [4] led to the development of alternative sensor systems that found application in clinical practice along with ion-selective electrodes. Fundamentally, an enzyme in close association with an electrode surface reacts with a substrate, and a product or co-substrate of the enzyme reaction is measured on the electrode. Clark described an electrode with an adjacent membrane sandwich containing GOx- an FAD-dependent oxidoreductase, which reduces oxygen as a co-substrate. Oxidation of glucose to gluconolactone and subsequent hydrolysis to gluconic acid causes a pH change that is measured with a pH electrode. Alternatively, the decrease of oxygen concentration, which is a consequence of glucose oxidation and reoxidation of the enzyme, can be determined with an oxygen electrode. Updike and Hicks developed a miniaturized oxygen sensor with GOx immobilized in a hydrogel and introduced a differential sensor construction that additionally measured ambient oxygen [56]. Notably, the YSI 23A Glucose Analyzer by Yellow Springs Instruments and the Beckman Glucose Analyzer became popular in clinical laboratories for glucose in plasma or whole blood. The Beckman Glucose Analyzer was introduced into the clinical laboratory in 1968 and used an oxygen electrode to measure the rate of decrease in dissolved oxygen when GOx was added to a sample. The YSI 23A combined the immobilized enzyme electrode with electrochemical hydrogen peroxide detection [31], which was traditionally measured exclusively photometrically using leuco dyes. Derivatives of the YSI 23A instrument remain popular for use as reference methods for evaluating consumer glucose monitoring systems. Disposable single-use sensors, based on electrochemical readout, were first introduced by MediSense (ExacTech), followed by Accu-Chek® Advantage by Boehringer Mannheim and FastTake® by Lifescan. Electrochemical sensors, configured to operate in a "reagent-free" mode [57], avoid reagent leakage and triggered the development of sensors for repeated glucose monitoring. Sensors for continuous (or repeated) glucose monitoring were directly derived from these reusable clinical enzyme sensors. In development almost since Clarke and Lyons' proposal, CGM products were launched successfully by Minimed, Menarini, Dexcom, and Abbott. Roche has a CGM system under development.

Electrochemical biosensor technology for glucose monitoring continues to attract not only the interest of the large commercial manufacturers of sensors but academic and clinical research groups. Many quality reviews of the status and progress of the technology can be found in the literature [58–67]. The details of operation, advantages and shortcomings, and prospects, and commercial considerations will be discussed hereunder. Different generations of electrochemical sensors will be introduced briefly. GOx-oxygen-hydrogen peroxide sensors came to be known as first-generation sensors [68]. The natural availability of ambient oxygen

makes first-generation technology useful for "reagent-free" analysis [57]. However, the low solubility and resultant low concentration of oxygen in biological fluids require measures such as sample dilution, diffusion barriers, or geometric arrangements [69] to maintain analytical performance. GOx can also be reoxidized by artificial electron acceptors, which themselves are reoxidized on an electrode. Organometallic complexes such as ferrocene [70], ferricyanide [71], and osmium complexes [59] are widely used. Small organic molecules, such as phenanthroline quinone and nitrosoaniline [71], have advantages for certain uses. These artificial electron acceptors are termed mediators. Sensors employing artificial mediators are referred to as second-generation sensors. The ability to use a high concentration of mediator improved the performance and manufacturability of sensors to the extent that they became viable consumer products [72]. When used with GOx, mediated sensors are subject to interference from varying concentrations of oxygen in the sample. Thus, glucose dehydrogenases, which are unreactive with oxygen, were introduced. PQQ-GDH, FAD-GDH, and NAD-GDH have all been used in second-generation sensors [71, 73], and significant work in enzyme engineering has gone into optimizing enzymes for specificity and stability [74–77]. The reduced cofactor of glucose-oxidizing enzymes can also be directly oxidized on an electrode surface, sometimes with the aid of surface modifications or small relays. Structured nanomaterials such as metal and metal oxides [63, 65, 78, 79] and carbon structures including graphene and fullerenes [64, 80, 81] have been reported, but are not yet in commercial use. Transfer through the heme group of cytochrome units of enzymes has been reported [82, 83]. Sensors according to this direct electron transfer technology are referred to as third-generation sensors. These generations are a convenient division, but not rigorous. The "wired enzyme" sensor of Heller et al. [59, 84] combines second-generation mediators with immobilization in a 2.5 generation sensor.

3 Self-Monitoring of Blood Glucose (SMBG)

Self-monitoring of blood glucose (SMBG) is the measurement of blood glucose values by a PwD for management of diabetes mellitus. SMBG is based on a "spot monitoring" method, where a singular blood glucose value is collected from a single blood collection, generally a finger stick. SMBG includes systems which are nonintegrated, where a single disposable test strip is used at a time, or somewhat integrated systems, where multiple test strips or test elements are included in a single meter. SMBG has become an essential tool for management of diabetes, especially for the patients using insulin therapy [9, 10, 85–101]. The utility of SMBG has also been established for patients who are not on insulin therapy and are instead using other means to regulate their blood glucose, such as oral or injected medications and regulation of diet/exercise [88, 89, 91–94, 97, 101–114], although there is no clear consensus on how often a PwD who is not using insulin should test [115–120]. The debate around the timing and effectiveness of SMBG for Type

2 (T2) is likely to continue as healthcare costs rise. As shown in the references above, through the monitoring of BG, improved outcomes can be observed with the lowering of HbA_{1c}, PwDs can gain awareness of postprandial blood glucose excursions, and effective behavioral modification and titration of medications can be monitored. SMBG systems, including requirements, system descriptions, measurement principles, sensor manufacturing, and system performance will be discussed in the following sections, concluding with an outlook for SMBG.

3.1 Requirements

3.1.1 Personal User Requirements

PwDs have different testing needs depending on the type of therapy they are undergoing. Generally, diabetes is classified into two categories, Type 1 (T1) and Type 2 (T2). A very general definition of the types is that T1 diabetes, formerly called insulin-dependent diabetes, is where the pancreas produces very little to no insulin to regulate glucose. In T2 diabetes (formerly called non-insulin-dependent diabetes), the PwD's ability to metabolize glucose is changed, either by developing insulin resistance or by reduced production of insulin. Therapy for T2 diabetes may vary significantly based on the progression of the disease in the PwD and ranges from oral medication, non-insulin injectables, and insulin therapy. A PwD who is undergoing insulin therapy will have more extensive testing needs than someone who is not undergoing insulin therapy. There is ever-increasing pressure to reduce the cost of treating diabetes, including the price of blood glucose monitoring supplies. The incidence of diabetes is rising, and the economic impact of diagnosed diabetes, direct and indirect, in the United States has been estimated at $245 billion in 2012, of which diabetes supplies (including testing supplies) are estimated to be 12% of that cost [121]. Consumers and payers are coming to regard diabetes meters and strips as commodity products [122], while the volume of SMBG systems sold has been increasing steadily, the price for SMBG systems has been decreasing. In addition, the lack of consensus of the utility of blood glucose monitoring in certain situations has led to many payers limiting or eliminating coverage of blood glucose monitoring supplies [115, 123]. The cost pressures on reimbursement for blood glucose monitoring systems has been a challenge for manufacturers as they strive to remain profitable and still introduce new technologies. The needs of the PwD have evolved over time beyond analytical performance requirements of the blood glucose measurement, varying with their condition; the needs of a T1 user are different from the needs of a T2 user. Even within the classification of T1 or T2, the type of therapy will also dictate the features that best suit the user.

3.1.2 Healthcare Provider Requirements

HCP requirements can be divided roughly into two categories: managing PwD data generated by SMBG systems during "home" use monitoring and managing systems at the POC. For the first situation, the SMBG system requirements are the same as described above in terms of system performance. For a HCP the ease of data management is more critical for POC systems. While many SMBG manufacturers have software packages that are able to download patient data from the meter, these packages rarely are able to download data from other manufacturer's systems. Consequently third-party systems (e.g., CliniPro®) may be required if a HCP must deal with multiple SMBG systems within a practice. The performance requirements for a POC blood glucose monitoring system for use at a HCPs facility or in a hospital are described in CLSI POCT12-A3 [37], and specifics of performance requirements will be covered in a later section. In addition to requirements around performance, systems for use in a POC setting must meet requirements for infection control and validation of meter cleaning and disinfection. CLSI POCT12-A3 [37], CLSI POCT13 [15], ISO15197:2013 [17], and the FDA draft guidance for POC BG systems [14] offer guidance around infection control and cleaning procedures for POC systems. Since the vast majority of POC meters are used across a certain population of patients, adequate controls around cleaning and disinfection procedures must be clearly defined, validated, and enforced to prohibit the transmission of blood-borne pathogens among the user population.

3.1.3 Payer Requirements

In general, third-party payers for healthcare services, such as governments, public insurers, and private insurers, are motivated to provide treatments to their subscribers that have been determined to be (cost-) effective [124] in comparison to standard care. Furthermore, decision makers do also consider the impact of the respective technology on their budgets.

The use of blood glucose monitoring by PwDs being treated with insulin has been demonstrated to reduce HbA_{1c} and the occurrence and costs of the associated comorbidities and is considered the standard of care for these patients [92, 124, 125]. Blood glucose monitoring for patients being treated only with oral medications can, in fact, facilitate long-term improvement in glycemic status, but only when the following conditions are met: The SMBG regimen is structured (both in timing and frequency) to obtain actionable information about each patient's glucose control. The data are generated and documented in a manner that facilitates analysis and discussion of glycemic patterns between patient and healthcare provider. Both the patient and the healthcare provider are well educated, willing, and skilled to make appropriate treatment decisions based upon the SMBG data. Both the patient and healthcare provider mutually agree upon treatment decisions and modifications.

This position mirrors the International Diabetes Federation's (IDF) guidelines for use of SMBG in non-insulin-treated diabetes. These guidelines recommend that SMBG should be used only when patients and/or their clinicians "have the knowledge, skills and willingness to incorporate SMBG monitoring and therapy adjustment into their diabetes care plan in order to attain agreed treatment goals." Unfortunately, these conditions for appropriate SMBG use are absent from the interventions used in many studies analyzed and summarized systematically by Clar et al. [126].

Structured SMBG as investigated in the STeP study is even more clinically relevant. The STeP study was a large prospective, cluster-randomized, multicenter trial evaluating the use of structured SMBG in 483 poorly controlled ($HbA_{1c} \geq 7.5\%$) insulin-naïve T2DM patients from 34 US primary care practices [127]. The primary endpoint was changed in HbA_{1c} over time. Patients in the structured testing group used a simple paper tool that facilitates collection and interpretation of 7-point glucose profiles over three consecutive days. These patients completed the tool on a quarterly basis, brought to the completed tools to medical visits, and discussed findings with their physicians. Structured testing group patients received training in BG measurement, including instructions for how to identify problematic glycemic patterns and how best to address such problems through changes in physical activity, portion sizes, and/or meal composition; structured testing group physicians received an algorithm describing various pharmacologic/lifestyle treatment strategies that could be used in response to the specific SMBG patterns identified. Active control group patients received enhanced usual care only and were instructed to use their meter following their physicians' recommendations but received no additional SMBG prompting, training, or instruction. At 12 months, intent-to-treat (ITT) analysis revealed that structured testing group patients ($n = 256$) experienced significantly greater improvement in mean HbA_{1c} than active control group patients ($n = 227$): -1.2% vs. -0.9%; $P = 0.04$. Per protocol (PP) analysis revealed an even greater HbA_{1c} reduction (-0.5%) in the experimental ($n = 130$) vs. control ($n = 161$) patient group (-1.3% vs. -0.8%; $P < 0.003$). Further analyses of data from the STeP study have revealed improvements in several other parameters, including clinicians' intensification of treatment, depression and diabetes-related distress, and patient self-efficacy and autonomous motivation in managing their diabetes. Similar findings were seen in a pilot study by Franciosi et al. [128] evaluating the efficacy of a structured SMBG-based intervention with T2DM patients treated with oral agents. Parkin et al. [110] have published a review article that provides more detailed descriptions of these studies. Still, reimbursement varies [129] to a large extent based on varying definitions of the interventions across the relevant studies and questionable assessment criteria by health technology assessment (HTA) agencies, e.g., the "somewhat arbitrary" [126] clinical relevance threshold of 0.5% which is often borrowed from the assessment of pharmaceuticals, but may hinder patients to materialize relevant results. Variability of reimbursement clearly raises similar questions such as clarity, suitability, and transparency of criteria used for reimbursement decisions as expressed recently

by the European Diagnostics Manufacturing Association (EDMA) position paper on health technology assessment for IVDs in the context of market access [130].

3.2 Monitoring Systems

The technology for SMBG is mature. Home-use tests for PwDs have been available in some form since the late 1970s [52, 131]. Early systems were based on testing of the user's urine, with the degree of color on the test strip indicating the glucose level. Today, urine glucose test strips are routinely used for screening both in hospitals and in general practice. The aim of screening is early identification of likely patients by examining large groups of the population. Patients with increased diabetes risk or PwDs can also benefit from urine-based glucose testing for screening purposes [132]. However, urine-based glucose testing is no replacement for blood glucose monitoring for several reasons. Measurements of blood glucose levels is not possible below the renal threshold, which usually corresponds to a plasma glucose level of 11.0 mM (198 mg/dL) [133], which, however, may vary during long-standing diabetes and pregnancy. Consequently no differentiation of hypoglycemia, euglycemia, and even mild hyperglycemia is possible. Further, the time lag between BG and urine levels may be very significant, depending on the time over which urine accumulates in the bladder [52, 134]. Due to these limitations of testing glucose in urine, there was a drive to produce home-use systems which were capable of testing blood and reducing the effect of the user's technique on the blood glucose results. These BG systems relied on photometric determination of blood glucose, which had many advantages over urine glucose monitoring. Electrochemical detection became more common in the 1990s. Improvements in SMBG, ease of use, accuracy, and precision continue to be made for both photometric and electrochemical blood glucose meters.

A SMBG system generally consists of an SMBG meter, disposable strips including a sensor, a lancing device, and one or more control solutions. Diabetes management software may also be included as part of the SMBG system. POC blood glucose monitoring systems designed for use in a clinical setting (hospital, clinic, or HCP facility) include more sophisticated data management, tracking, and transfer capabilities than a consumer system. Currently, the personal user is able to choose from a large number of systems which provide a competitive set of features. Users have come to expect some features as part of a standard user experience, such as small blood volume, fast test time, and some data storage capability. Other features are becoming more common in the competitive blood glucose monitoring market, such as diabetes management tools [135, 136]. The majority of systems currently on the market require sample volumes less than 1 μL, with some systems requiring sample volumes as low as 300 nL. Test time has also decreased significantly since the introduction of consumer SMBG meters. Test times of five seconds are now common. Meters can be differentiated by the availability of additional features, as well as through system performance. One of the most attractive

additional user features is the so-called no code. Instead of the end user having to enter a lot-specific code or insert a code key, the user is able to simply use the strips directly from the vial. Advances in strip manufacturing processes and measurement techniques enable either universal coding, where the strips are produced reproducibly enough to require the same code for all lots, or non-evident coding, where the appropriate code is transferred from the strip packaging or from the strip characteristics. With either method, the end user is not required to input code-specific information into the meter, improving the ease of use of the system and reducing user-induced measurement error [137–139].

Another characteristic which improves ease of use and reduces opportunities of error [140, 141] is the integration of multiple tests into one single container, thus removing the need to handle single strips. A single container may contain 10 to 50 blood glucose tests, depending on the manufacturer of the system. The lancet for this type of system may be integrated into the body of the meter or attached to the body of meter. The advantage of integrated systems is the reduction of the number of steps the PwD must take to complete a blood glucose test, with a possible reduction of errors introduced by multiple handling steps. The Accu-Chek® Mobile is an example of a fully integrated system [140], with a 50-test cassette, attached lancing device with a drum of six lancets, automatic coding, and small required sample size.

Advanced SMBG systems offer features for enhanced diabetes management. The majority of meters currently on the market are able to store multiple BG measurements. More advanced meters will provide average blood glucose measurements at preset intervals. In addition, meters such as the Accu-Chek® Aviva Plus and Accu-Chek® Nano include before and after meal marking, before and after meal BG averages, as well as a customizable hypo indicator, customizable test reminders, and post-meal test reminder. Beyond memory functions, test averaging, and reminders, some meters include features which provide features useful to the insulin-dependent PwD. The Accu-Chek® Aviva Expert BG system provides the user the ability to record events related to diabetes management, such as blood glucose test results, carbohydrate intake, bolus and basal insulin delivery, and significant health events (e.g., exercise, stress, illness). In addition, the Accu-Chek® Aviva Expert and Accu-Chek® Insight systems have a built-in bolus advisor, which provides the user with an insulin dosing or carbohydrate intake recommendation to correct BG levels that are not in the targeted glucose range. Features such as these, especially bolus calculators [142–144], are useful for the insulin-dependent PwD.

Integration of a SMBG system with an insulin pump is the next level of combining SMBG with diabetes management solutions. In these systems, the meter and the remote control for the pump are often the same device. In the Accu-Chek® Combo system, the Accu-Chek® Aviva Combo meter is the remote control for the Accu-Chek® Spirit Combo insulin pump, as well as a BG meter which includes data management features, such as a log book, trending graphs, and reports; other features include event-based reminders and the ability to enter specific data regarding an event (illness, stress, exercise, etc.). The new feature

for bolus advice in the Combo system uses SMBG test results directly from the meter in the bolus calculator, and the user can use the meter/pump remote to deliver the bolus. These features are useful for the PwD ([142] and references contained within), especially for pediatric PwDs [145].

SMBG systems are often coupled to software packages or online tools produced by the manufacturer of the system to allow for more in-depth analysis of SMBG data collected by the user [66, 146, 147]. SMBG data is transferred from the meter to the software package. Both consumers and healthcare providers can use the reports available to help fine-tune therapy and behavior modification for PwDs. These software applications are available on primarily PC systems, although online applications are being introduced. Accu-Chek® 360°, a powerful diabetes management tool, is available for both PC and as a diabetes management app for Android smartphones. Accu-Chek® Connect Online is a web-based diabetes management portal which allows downloading of SMBG data from all Accu-Chek® devices [146]. This product allows for easy sharing of data between PwDs and HCPs/caregivers. The 510(k)-cleared Accu-Chek® Connect Diabetes Management app allows for wireless data transfer from the Accu-Chek® Connect meter to smartphones for data analysis and for bolus calculations.

3.2.1 Measurement Principles

Active Components

Commercially available SMBG systems are based on optical or electrochemical detection schemes. For both schemes, enzymes provide the specificity for glucose. Except for the actual signal transduction, the mechanism for signal generation is very similar for optical and electrochemical systems. Glucose sensors use oxidoreductase enzymes which oxidize glucose to gluconolactone. The commonly used enzymes for SMBG systems are GOx (EC 1.1.3.4), quinoprotein glucose dehydrogenase (PQQ-GDH, EC 1.1.5.2), FAD-dependent glucose dehydrogenase (FAD-GDH, EC 1.1.99.10), and NAD-dependent glucose dehydrogenase (NAD-GDH, EC 1.1.1.47). GOx and FAD-GDH have the same cofactor, flavin adenine dinucleotide, with the native and preferred final electron acceptor for GOx being oxygen. FAD-GDH, unlike GOx, does not use oxygen as the final electron acceptor. PQQ-GDH uses pyrroloquinoline quinone as its cofactor and NAD-GDH nicotinamide adenine dinucleotide. FAD and PQQ are bound to their respective enzymes through strong interactions, while NAD is not bound permanently to NAD-GDH [73]. A generic reaction scheme for electrochemical biosensors is shown below in Fig. 1.

Each of these enzymes has advantages and disadvantages, and the properties of the enzyme will vary with the specific parent organism of the enzyme and depend on potential protein engineering of the enzyme. A brief overview of the enzymes commonly used in SMBG is provided below. An excellent review of enzymes used

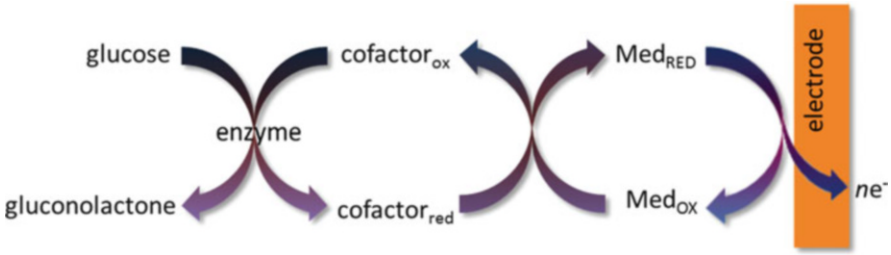

Fig. 1 Generalized working electrode reaction scheme for a SMBG biosensor. "ox" is the oxidized form of the cofactor or mediator, "red" is the reduced form of the cofactor or mediator, and n is the number of electrons (e^-)

for glucose biosensing has been written by Ferri et al. [73]; the reader is referred to that review for more information.

GOx was the first enzyme to find widespread use in glucose sensors [4, 31, 52, 71, 73]. The parent organism for GOx is Aspergillus niger, with the majority of the GOx in use today for glucose sensing produced from this organism. GOx has excellent specificity for glucose, with some interference from 2-deoxy-D-glucose and minimal interference from galactose. These sugars are not generally considered significant for most SMBG use; labeled 2-deoxy-D-glucose may be used for PET-scanning and is not generally encountered by users except in very controlled circumstances [148], and galactose interference is a problem for mainly POC use when galactosemia is present in neonates [33, 149, 150]. Oxygen is the native electron acceptor for GOx, producing hydrogen peroxide as a result of the catalytic reaction. Generally, detection of the hydrogen peroxide produced when oxygen is the electron acceptor is not robust in disposable glucose sensors used for SMBG, making the use of a mediator to transfer electrons to the electrode surface necessary for efficient signal generation. The reduced FADH2 cofactor is fairly tolerant of mediator identity, accepting many metal complexes or organic mediators, as long as the redox potential of the mediator is suitable. When a mediator is used for a GOx-based sensor, oxygen interference becomes a factor and is the main reason why GOx is no longer used for SMBG sensors produced by the main four SMBG manufacturers.

FAD-dependent glucose dehydrogenases, FAD-GDHs, do not use oxygen as its native electron acceptor, unlike GOx. There is variability in the sugar specificity in FAD-GDHs, depending on the parent organism [73]. For FAD-GDH derived from bacteria, an integrated electron acceptor is part of the enzyme in the form of cytochrome c-containing subunit. This FAD-GDH lacks specificity for glucose, with substantial activity for maltose. More commonly used are FAD-GDHs derived from fungal parent organisms. FAD-GDHs from fungi do not have the integrated acceptor, but instead are able to use a wide range of mediators as long as the redox potential of the mediator is suitable. Sugar specificity is, in general, excellent for FAD-GDH derived from fungi; however, there is substantial activity toward

2-deoxy-D-glucose and xylose. Since FAD-GDH does not accept oxygen as an electron acceptor, sensors with FAD-GDH do not experience interference from variations in oxygen partial pressure or have altitude limitations for use as seen in GOx-based systems.

PQQ-GDH (PQQ-dependent glucose dehydrogenase)-based SMBG sensors use soluble PQQ-GDH, derived from *Acinetobacter calcoaceticus*. It has excellent catalytic activity, allowing for fast glucose sensing; however, native PQQ-GDH can have interference from several sugars, including maltose, galactose, and xylose. With protein engineering, mutant forms of PQQ-GDH have been made with excellent glucose specificity, reducing the impact of maltose to the point where significant interference is not observed [73, 151–158]. PQQ-GDH does not have oxygen interference and is able to accept a wide variety of mediators, both organic and inorganic metal complexes.

NAD-dependent glucose dehydrogenase, NAD-GDH, generally has good substrate specificity, but its cofactor is not bound and must be included as part of the strip reagent matrix as an additional component. Also, the number of effective mediators for NAD-GDH-based systems is more limited due the specific requirements for rapid reaction of the mediator with NADH (e.g., quinones and quinoid compounds).

Direct oxidation of the reduced cofactor is not generally possible for GOD, PQQ-, and FAD- dependent enzymes. In theory, NADH could be oxidized directly at an electrode surface; in practice, direct oxidation of NADH often results in incomplete regeneration of active NAD and electrode surface fouling [159–161]. To overcome difficulties with direct electron transfer, all commercially available SMBG systems use a mediator system for transferring the electrons from the reduced cofactor to the electrode surface. The reduced mediator is oxidized at the electrode surface, where the current generated or the charge collected is directly related to the amount of glucose in the sample. FAD- and PQQ-dependent enzymes are relatively tolerant of the mediator choice as long as a mediator with suitable potential is chosen. NAD-dependent enzymes are far "pickier" with respect to effective mediators due to the need for hydride transfer between NADH and the mediator. For this reason, quinones and quinoid compounds, such as quinone diimines, phenazines, and related dyes, are effective mediators for NAD-based systems [162, 163]. Regardless of the identity of the mediator used in SMBG systems, freely diffusing mediators are the norm. Table 1 shows some examples of enzyme and mediator combinations used in SMBG products.

Measurement Methods

The vast majority of electrochemical SMBG systems rely on amperometric detection, with coulometric detection used in a small number of systems [52, 61, 66, 71, 131]. For both of these measurement methods, the general mechanism for signal detection is the same; the reduced mediator produced from the reaction of the reduced cofactor of the glucose-converting enzyme and oxidized mediator must

Table 1 Non-exhaustive list of examples of enzymes and mediators used in SMBG systems

Enzyme	Mediator	Product examples
GOx	Hexacyanoferrate (III)	OneTouch® Ultra®
	Hexaamineruthenium (III)	iBGStar®
FAD-GDH	Hexacyanoferrate (III)	One Touch® Verio®
	Phenothiazine	Bayer Contour® Next
	Osmium based	Abbott FreeStyle Lite®
PQQ-GDH	Not indicated	Nipro TRUE2go®
Mut-Q (engineered PQQ-GDH)	Nitrosoaniline	Roche Accu-Chek® Aviva Roche Accu-Chek® Performa
NAD-GDH	Phenanthroline quinone	Abbott FreeStyle Neo®

diffuse to the test strip working electrode surface, where it is oxidized. The amount of oxidation current or charge collected is directly related to the amount of glucose in the sample. In a classical amperometric experiment, there are three electrodes used in the cell for evaluating the current: a working electrode, where the reaction of interest takes place; a reference electrode, against which the potential applied to the working electrode is set; and a counter electrode, where the current flows between the working and the counter electrode. No current flows through the reference electrode, ensuring that a stable potential is maintained. The most common reference electrode type is a silver/silver chloride reference. This type of electrochemical cell can be expensive and is not often used in SMBG test strips. Instead, most SMBG strips use a two-electrode configuration for the primary electrode set, with a working electrode and a counter electrode. This counter electrode serves as a reference electrode against which a potential difference between the working and counter is applied. The current which flows between the working and the counter electrode is measured. For some SMBG strips, the counter electrode is coated with Ag/AgCl and functions much like a traditional reference electrode, maintaining a stable potential as long as the amount of current passed through the electrode does not disrupt the reference system. More commonly, SMBG strips are configured for use in a "biamperometric" measurement, where the counter electrode reaction also serves as the reference reaction. In this configuration, the absolute potential of the system is not stable and will drift according the properties of the system, in particular, the glucose concentration. The impact of not having a stable reference electrode is accommodated by careful design of the measurement method to ensure that reproducible measurements are made. While the relationship between glucose in the sample and the current produced is, in principle, a very simple and direct relationship, there is considerable variation in the magnitude of the current response due to the influence of temperature on diffusion. The change in diffusion speed is approximately 2% for a 1K change in temperature. Hematocrit also has a large impact on the response, since red blood cells in the sample influence the diffusion of glucose in the sample. Correction of the primary current response for the influence of temperature and hematocrit is accomplished in

different ways, depending on the SMBG system in question. Given the extremely competitive nature of the business of SMBG, different manufacturers strive to keep the exact natures of their measurement methods and correction algorithms confidential. Basic information about the correction mechanisms for BG values can be inferred from product and patent literature. However, the finer details of the compensation algorithms are held as trade secrets.

Temperature correction, in its simplest form, can be achieved by introducing a correction factor based on the measurement of the temperature experienced by the meter. While this type of correction is elegant in its simplicity, BG measurements which have been corrected in this manner can suffer from over- or under- compensation due to differences in the temperature measured by the meter and the temperature of the reaction on the test strip. When a system with a meter-based temperature measurement correction is in a rapidly changing temperature environment, for example, when the meter has been moved from a warm environment to a cool environment and a measurement is made without letting the meter equilibrate to the ambient temperature, a bias in the BG result may occur due to overcompensation for high temperature perceived by the meter. For systems using a meter-based temperature measurement, a waiting time needs to be defined to allow for the system to equilibrate. A more sophisticated temperature compensation scheme relies on the sample properties measured on the test strip, as in the Accu-Chek® Aviva and Accu-Chek® Performa meters. In this compensation method, electrochemical impedance measurements are used to monitor changes in the conductivity in the sample due to temperature in the sample measurement zone. These impedance measurements are used in the correction algorithm to compensate for the impact of sample temperature on the BG response [164, 165].

Compensation for hematocrit is achieved through several methods, again depending on the manufacturer. In the Accu-Chek® Advantage system, hematocrit correction was achieved through monitoring the shape of the current response over time [71]. More current BGM systems use other means of correcting for hematocrit interference; for example, some systems will use a secondary electrode as a hematocrit sensing electrode, using the response of the secondary electrode to correct the primary glucose current measured on the working electrode. Other systems use a technique the manufacturer refers to as "Dynamic Electrochemistry®," where the response of the working electrode to a varied potential input is used to correct for hematocrit [166, 167]. Roche's Accu-Chek® Aviva and Accu-Chek® Performa systems use electrochemical impedance measurements made with the working and counter electrodes of the test strip to measure the impact of hematocrit on the sample conductivity. The impedance measurements used in the correction of temperature are also used to correct for hematocrit interference in that same family of products [164, 165].

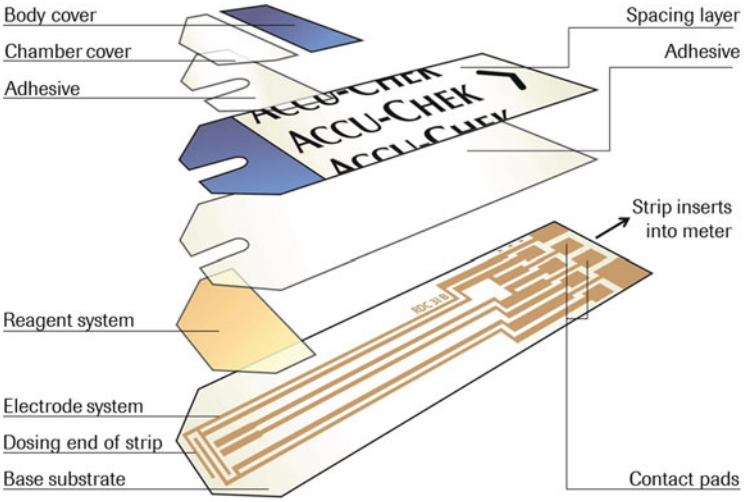

Fig. 2 Accu-Chek® Aviva Test Strip Architecture. Exploded view showing the base film and electrodes, spacer to define the electrode area, and cover layer hydrophilic to define the sample chamber

Correction of other interferences can also be achieved through the measurement method, selection of mediator, or a combination of the two. By choosing the applied potential and/or mediator carefully, interference from some electroactive substances, such as uric acid and acetaminophen, can be avoided [59, 61, 71]. Other methods for reducing endogenous and exogenous interfering substances have been suggested [61]; however, implementing these approaches in inexpensive, disposable SMBG test strips has not been feasible.

3.2.2 Sensor Manufacturing

In the sections below, a brief overview of electrochemical SMBG sensor manufacturing processes is outlined. Since there are a wide variety of SMBG sensors on the market with different sensor architectures, reagents, and manufacturing techniques, an exhaustive review is outside the scope of this description. As an example, a view of the Accu-Chek® Aviva test strip is shown in Fig. 2 [71].

The base film in commercially available SMBG sensors is commonly a thin thermoplastic substrate, usually made of polyester. Depending on the nature of the processing steps, the base film, or "web," may be a large sheet (discrete processing) or a continuous roll (reel-to-reel processing). The benefit of reel-to-reel processing is in the speed inherent in web converting processes, which allows for cost benefits to be derived from large-volume production [59, 61].