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Electrochemical Non-enzymatic Glucose Sensors: A Perspective and an Evaluation

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An overview of glucose sensors is presented, with specific focus on the promise of non-enzymatic electrochemical glucose sensors. The review addresses their merits and shortfalls with respect to their commercially available enzymatic counterparts. The mechanisms for catalysis are evaluated, and the future of the systems discussed.

Keywords: glucose, biosensor, non-enzymatic sensing, electrochemical sensing, electrocatalysis, amperometric sensors

1. INTRODUCTION

The clinical conditions of diabetes mellitus are well known and well understood, yet remain a growing concern as the prevalence of the disease increases worldwide at an alarming rate. A number of life-threatening and life-impeding conditions greatly affect the diabetic community, resulting in a much greater risk of cardiac, nervous, renal, ocular, cerebral and peripheral vascular diseases. It was estimated that 2.8 % of the world population was affected by diabetes in 2000, approximately 171 million people [1]. By 2030 however, this is projected to more than double, with 366 million people suffering from the disease globally [1]. Treatment has become a far more sophisticated science, with self-testing becoming increasingly more compact and accurate, and continuous glucose monitoring now obtained from practical commercial sensors. Glucose sensors are therefore a highly active area of sensor research, and accordingly they account for approximately 85% of the biosensor industry [2].

Sensitive and selective glucose sensors are not only relevant for use in blood sugar monitoring, but also in the food industry, bio-processing and in the development of renewable, sustainable fuel cells. Non-enzymatic glucose electrodes used in direct oxidation show considerably greater

sensitivity, with oxidation currents as high as mA mM^{-1} cm^{-2} now being reported [3]. However, the superior selectivity and relative non-toxicity of enzyme based electrodes has retained the focus of commercially available glucose sensors on enzymatic systems.

Over the past decade the development of non-enzymatic glucose sensors has risen at a considerable rate. The fabrication of a wide variety of nanomaterials has introduced a plethora of selective and highly responsive glucose sensors. In 2005 Park et al [4] published an excellent and authoritative review regarding the development of non-enzymatic glucose sensors. Yet, in the 5 years since, over 80 glucose sensors have been reported (Tables 1, 2 and 3), and a further 60 publications report possible non-enzymatic materials that may be developed and used for glucose sensing. Research into this area of sensing is therefore evidently in full force, and in this review we hope to assess the current situation with respect to the practical application of non-enzymatic glucose sensors we note. In 2008 two extensive critical reviews of *enzymatic* electrochemical glucose sensors were published by Heller and Feldman [5] and by Wang [2]. As such, enzymatic systems will be briefly discussed herein, but the reader is encouraged to read the latter two reviews for more substantial understanding and detail.

2. ENZYMATIC GLUCOSE SENSORS

Enzymatic glucose sensors dominate the biosensing industry, particularly with the ever advancing development of self-testing and continuous blood glucose monitoring. The first enzymatic glucose sensors were introduced by Clark and Lyons in 1962 [6], in which oxygen consumption was monitored based on the catalytic oxidation of glucose in the presence of oxygen. Problems with varying amounts of background oxygen led to further development of the oxygen sensor by Updike and Hicks [7] which corrected for background oxygen levels. The first amperometric enzyme glucose sensor was developed in 1973 [8], in which the anodic production of hydrogen peroxide was analysed instead of the highly variable oxygen reduction current. Since this ground work the field of glucose sensors has progressed rapidly with increasingly innovative, efficient, and superior electrochemical technology being developed every year.

Originally, and until relatively recently, the enzyme glucose 1-oxidase (GOx) was the main catalytic component used in enzymatic biosensors. It was described as the 'ideal enzyme' for glucose oxidation in the review by Wilson and Turner [9] in 1992, owing to a relatively high selectivity, sensitivity and stability, compared to other enzymatic materials. The key component of the large protein molecule is the redox centre, flavin adenine dinucleotied (FAD). The flavin group is reduced on interaction with glucose, thus producing the redox product of glucolactone, in accordance to equation 1.

$$GOx(FAD) + glucose \longrightarrow GO_x(FADH_2) + glucolactone \quad (1)$$

This redox centre is deep within the enzyme however, protected by a thick protein layer. As such electron transfer to the active centre is a major limiting factor, and the cause for complicated

electron transfer mechanisms. To date, three approaches are in place to oxidise the reduced $FADH_2$ centre, catalytically regenerating it either by reducing naturally present oxygen, reacting with a mediator, or direct oxidation by the electrode. As such, enzymatic glucose sensors are discussed with respect to their regeneration mechanism, as will be observed in the following sections. They are also summarized in Figure 1.

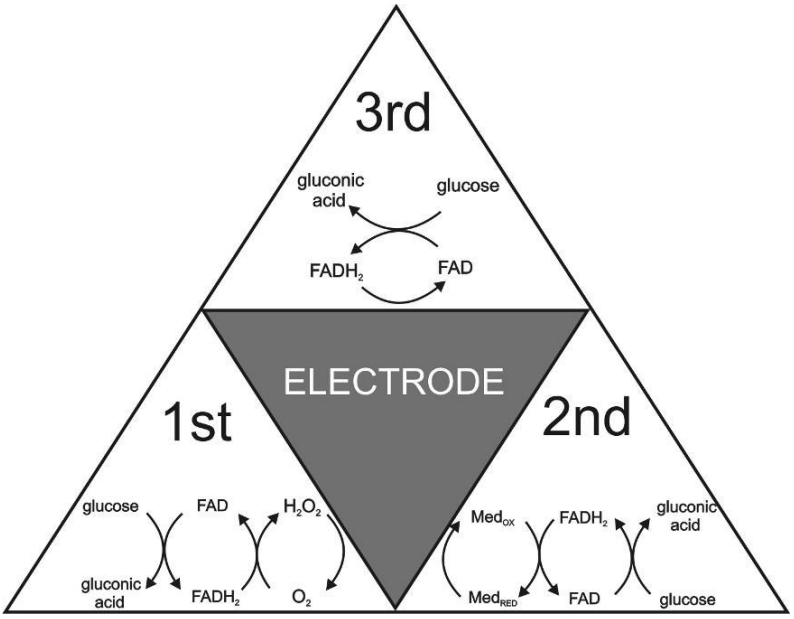


Figure 1. Summary of enzymatic glucose oxidation mechanisms, presented as first, second and third generation sensors

2.1. First generation enzymatic glucose sensors

First generation glucose biosensors are dependent on the presence of oxygen as a co-substrate to ensure the catalytic regeneration of the FAD centre. The consequent reaction is shown in equation 2, and is the immediate process to follow equation 1 above.

$$GOx(FADH_2) + O_2 \longrightarrow GOx(FAD) + H_2O_2 \quad (2)$$

The original Clark oxygen electrode used depletion of oxygen as a guide to glucose oxidation, the approach used in the fabrication of the first commercial glucose biosensor by Yellow Spring Instrument Company in 1975. Two years prior to this however, an amperometric approach to glucose determination was proposed by direct measurement of the hydrogen peroxide produced [8]. This offered a more simple, precise and selective approach to blood glucose testing as interference by background oxygen was eliminated, and the sensing of hydrogen peroxide at the anodic potential of 0.6V vs. SCE, gave a current directly proportional to the glucose concentration. The oxygen was regenerated in the oxidation of the peroxide, thus replenishing the oxygen electron mediator.

First generation glucose electrodes face two major problems however; the presence of electroactive interference species in the blood and the dependence on free oxygen as a catalytic mediator. The former problem is addressed more thoroughly in Section 4 of this review, but in short the potential range at which hydrogen peroxide is oxidised coincides with the oxidation potential of numerous compounds found in blood. These are listed in Table 4, alongside their normal human blood levels. Furthermore, the frequent use of a modified platinum electrode leaves the sensor open to fouling by blood proteins and adsorptive inhibition by chloride ions. As such various approaches to avoid interference effects have been undertaken and these are discussed later on.

The oxygen dependence of first generation enzyme glucose sensors is a problem specific to this generation. The errors surrounding this high dependence on oxygen to mediate regeneration of the catalytic centre are quite significant, as oxygen levels can vary considerably. There is an oxygen limitation in which there is quite simply not enough oxygen available in a real blood sample to efficiently maintain glucose oxidation, thus this oxygen deficit has a great impact on accurate determination of glucose levels. A number of ways to address this deficit have been suggested and demonstrated, yet all involve overcomplicating the fabrication method and enlarging the overall sensor. Approaches include the use of a two-dimensional cylindrical electrode which doubles the oxygen input relative to the glucose input [10], the use of films that limit specific diffusion, allowing more oxygen relative to glucose through the film [11], and also the use of oxygen rich electrode materials which act as an internal source of oxygen for the reaction [12,13].

2.2. Second generation enzymatic glucose sensors

Due to the major problem of oxygen dependence observed in first generation enzymatic glucose sensors, the use of alternative co-substrates was introduced. Synthetic, electron-accepting mediators are utilised to facilitate electron transfer, with their consequent re-oxidation by the electrode resulting in a quantifiable amperometric current. A number of non-physiological mediators have been reported including ferrocene derivatives [14,15] and ferricyanide [16] of which most commercial sensors use, quinones [17,18] and transition-metal complexes [19]. Each of these mediators posses a number of essential attributes that idealise them for enzymatic glucose analysis. These include a low molecular weight and insoluble nature so as to effectively diffuse without complexing, a reversible or quasireversible properties, a suitably lower redox potential to avoid oxidation of interfering species, a high stability and resistance to forming side compounds, and a low toxicity (particularly where in vivo use would be required).

Unfortunately, problems still remain when using a mediator. Being small, diffusive molecules, maintaining the presence of the mediator near the electrode and enzyme surface is very difficult, particularly over relatively prolonged use, thus requiring elaborate and complicated methods of tethering the mediator to the two entities [16--20]. Although the mediator ideally reacts with the enzyme at a considerably faster rate than oxygen, the possibility of dissolved oxygen also competing with the mediator is highly likely, thus reducing the efficiency of the system and causing a build up of

hydrogen peroxide. It is also possible for the mediator to react with interference species present in the blood, further affecting the accuracy and efficiency of the analytical system.

In recent years, the most innovative enzymatic glucose sensor to have been developed is that developed by Heller et al with Abbott Diabetes Care [20].

The FreeStyle blood glucose monitoring system uses glucose dehydrogenase (GDH) as the enzyme as opposed to GOx, and an osmium redox mediator. The mediator is chosen in particular due to its substantially negative formal potential of 0 to -0.2 V vs. Ag/AgCl, thus allowing a system of two parallel facing working and counter electrodes merely 50 μm apart. The oxidised Os^{3+} is thus unable to be reduced by the positively poised Ag/AgCl counter electrode, and current does not pass between the two electrodes directly.

As such an astonishingly small sample size of 0.3 μL may be used, and effective bulk electrolysis of the glucose is achieved [20]. The continuous glucose monitoring system, FreeStyle navigator, more recently commercialised in 2007, utilises a very new patented technology of wired enzymes [20--22]. GOx is once again selected as the oxidising enzyme, and is 'wired' to the electrode surface using a redox hydrogel consisting of a polymer bound osmium redox mediator. It is a non-diffusive approach which addresses one of the main issues of second generation enzymatic glucose sensors [20].

2.3. Third generation enzymatic glucose sensors

Third generation enzymatic glucose sensors involve direct electron transfer between the enzyme and the electrode, without the need for natural or synthetic mediators. This is an ambitious type of enzymatic glucose sensor, yet in recent years it has become increasingly realistic. Achieving mediator free electron transfer between the electrode and redox enzyme is of great fundamental interest. It would be a more perfect system, as the complications of tailored mediators would be avoided, and selectivity and sensitivity could be very high and unhindered.

As mentioned previously, the biggest difficulty in achieving direct electron transfer between the electrode and enzyme is the thick protein in which the redox active centre is embedded. Mediators and glucose access this centre by means of diffusion and penetration of the 3D molecular network, yet this is not possible at a perfect planar electrode. In recent years however, rapid advancement in the development of nano and porous materials have grossly increased electrode surface areas and dynamics [23], [24,25]. Mesoporous electrode materials are of particular interest [24,25], offering an electrode surface that entraps and encompasses the enzyme. As such direct electron transfer from enzyme to electrode can occur, and a current corresponding to enzyme oxidation may be directly observed, without the complications of mediators, the intrusion of electroactive interferences, or the dependence on dissolved oxygen. This does not necessarily resolve the interference effects of dissolved oxygen competing with the electrode to regenerate the enzyme, but the impact can be considerably lower.

Other approaches reporting the mediator-free oxidation of GOx and other enzymes include the use of an over-oxidised boron doped diamond electrode (BDDE) [26] in which the surface carboxyl

groups covalently bond to the enzyme through glutaraldehyde, over-oxidised poly(pyrrole)/tetrathiafulvalene-tetracyanoquinodimethane (TTF-TCQN) salts in a tree-like crystal growth on a platinum electrode [27], and an electro-conductive screen printed polymer membrane [28], though the latter may be deemed a second generation enzyme electrode given that the poly(pyrrole) essentially mediates electron transfer.

2.4. The advantages and disadvantages of enzymatic glucose electrodes

Overall, enzymatic glucose sensors remain a balance of advantages versus disadvantages of which both are significant. Despite dominating the glucose sensor industry, enzymatic systems have a number of critical flaws. First generation sensors suffer a high oxygen dependency, and are therefore immediately unsuitable for practical and reliable analytical use.

Furthermore, their sensory ability is highly impacted by the presence of other electroactive interferences in the sample that are always commonplace in real blood samples. To overcome this, second generation sensors have utilized synthetic mediators as opposed to oxygen, achieving oxygen independence and a lower amperometric potential thus avoiding some electroactive interferences. In compensating however, the electrodes have become even more elaborate, and complicate mass production of a reproducible sensor. Finally, third generation sensors are still in their infancy, yet evidence for working based on nano mesoporous electrode surfaces shows some promise [23--25].Stability issues that surround enzymatic systems in all fields of science also hinder the development and application of enzymatic glucose sensors. Despite numerous attributes with respect to relative stability, GOx is still constrained to pH ranges of 2-8, temperatures below 44ºC [9,29,30], and ambient humidity levels [29,30]. Ensuring the stability of immobilized enzyme and mediator electrodes requires considerable attention, with elaborate fabrication processes of electropolymerisation [30], covalent cross-linking at a pretreated surface [31], sol-gel entrapment at the electrode surface [32], or even the electrochemical 'wiring' of enzymes to mediated polymer chains [20--22], ensuring greater fabrication costs lower reproducibility, and short-term stability. As diabetes continues to rise in developing countries, such high cost fabrication processes and short shelf-life of sensors become less viable. Despite these problems, enzymatic glucose sensors remain commercially unchallenged. This is understandable given the high selectivity of the enzyme towards glucose. Fabrication by mass production has been possible, allowing for single-use disposable electrodes, due to the possibility of screen printing electrodes. Screen printed carbon electrodes are produced from a complex mixture of mediators, enzyme and conductive carbon with binders and stabilizers, such that the mediator is bound to the electrode surface along with the enzyme, and can still mediate electron transfer. The method was introduced in the 1990's by a number of groups [33,34].

3. NON-ENZYMATIC GLUCOSE SENSORS

The use of non-enzymatic electrodes as glucose sensors potentially promises a fourth generation to analytical glucose oxidation. Instead of facilitating the needs of a fragile and relatively difficult enzyme however, non-enzymatic electrodes strive to *directly* oxidise glucose in the sample.

This is an ideal system that was first investigated a century ago by Walther Loeb [35] who electrochemically oxidised glucose in sulphuric acid at a lead anode. This occurred long before to the fabrication of the Clark oxygen electrode [6], though extensive research into the non-enzymatic approach actually coincided with enzymatic development [36, 37]. Despite decades of research into non-enzymatic systems however, the problems associated with this approach have prevented the practical application of the sensors.

Table 1. Non-enzymatic glucose sensors that have only used fixed potential, chronoamperometric methods for electrochemical determination. The table is presented with respect to electrode type, sensitivity, linear range, limit of detection and publication year. Abbreviations are given at the foot of the table.

Electrode composition	sensitivity (mA mM^{-1} cm^{-2})	Linear Range (mM)	LOD (µM)	Year	Ref.
POROUS LAYERS					
Ni film Au nanoporous	5.1	$1x10^{-3}$ - 10	not given	2009	[108]
Pt nanoporous	0.642	0.1 - 1.5	not given	2008	[53]
Pt nanoporous	0.291	1.0 - 10	not given	2008	[69]
Pt-Ir nanoporous	0.0937	1.0 - 10	not given	2008	[76]
Au 3D GFE[a]	0.0466	$5x10^{-3}$ - 10	3.2	2008	[81]
Pt nanoporous	0.0375	0.05 - 30	50.0	2008	[71]
Au porous	0.032	2.0 - 20	2.0	2007	[83]
Pt InvOp[b]	0.0313	$1x10^{-3}$ - 10	0.1	2005	[68]
Au macroporous film	0.0118	2.0 - 10	5.0	2007	[82]
C OM[c]	0.0108	2.5 - 5.0	20.0	2007	[186]
PtPb nanoporous on Ti	0.0108	1.0 - 16	not given	2008	[77]
Pt nanoporous	0.0017	1.0 - 10	97.0	2007	[72]
Pt nanoporous	0.00083	not given	not given	2008	[70]
NANOTUBES					
Pt-NC[d]-CNTs[e]	0.28	$2x10^{-4}$ - 12	0.2	2009	[55]
Cu nps/SWCNTs/GC	0.256	$5x10^{-4}$ - 0.5	0.3	2004	[59]
C SWCNT films	0.2486	0.01 - 2.16	10.0	2007	[158]
Pd-SWNTs	0.16	0.5 - 17	0.2	2009	[244]
Ni (II)-Qu[f]-MWCNT-IL[g]	0.0369	$5x10^{-3}$ - 2.8	1.0	2009	[60]
CuS2-nanocrystal-CNTs	0.035	$1x10^{-3}$ - 12	1.0	2009	[55]
MnO2/MWNTs	0.03319	0.01 - 28	not given	2008	[160]
PtRu/MWNTs	0.02826	1.0 - 15	25.0	2008	[56]
PtPb nps/MWCNT	0.0178	up to 11	1.8	2007	[57]
Cu-CNTs-GCE	0.01776	$7x10^{-4}$ - 3.5	0.2	2007	[163]
Pt/MWCNTs	0.01183	1.0 - 9 & 9 - 23	50.0	2008	[54]
PtRu/MWNT–IL/GC	0.0107	0.2 - 15	50.0	2009	[128]
SnO2-NC-CNTs	0.0099	$3x10^{-3}$ - 12	3.0	2009	[55]
CuS nanotubes	0.00784	0.05 - 5.0	not given	2008	[245]
Cu2O/MWCNT	0.00653	$5x10^{-5}$ - $5x10^{-3}$	0.1	2009	[58]
C MWCNTs	0.00436	$2x10^{-3}$ - 11	1.0	2004	[157]
Au NTA [h]	0.00113	1.0 - 42.5	10.0	2009	[92]
Pt-NTA	0.0001	2.0 - 14	1.0	2005	[75]
Pt-MWCNTs	not given	1.0 - 26.5	not given	2007	[166]
CoOx/MWCNT/GC	not given	0.15 - 5.0	70.0	2008	[167]

NANOPARTICLE					
Ni-BDD	1.04	0.01 - 10	2.7	2010	[106]
Ni nps on CFP [i]	0.4204	$2x10^{-3}$ - 2.5	1.0	2009	[107]
Cu nps/SWCNTs/GC	0.256	$5x10^{-4}$ - 0.5	0.3	2004	[59]
Ni mod CILE [j]	0.202	0.05 - 23	6.0	2009	[112]
Au nps ITO [k]	0.1835	$4x10^{-3}$ - 0.5	5.0	2009	[86]
Au nps on 3DSN [l]	0.179	$7.5x10^{-5}$ -$7.5x10^{-4}$	0.1	2006	[87]
Pt nps	0.1377	0.2 - 3.2	5.0	2007	[73]
Pt-xGnP [m]	0.0615	up to 20	1.0	2008	[74]
CuS2-nanocrystal-CNTs	0.035	$1x10^{-3}$ - 12	0.0	2009	[55]
PtPb nps/MWCNT	0.0178	up to 11	1.8	2007	[57]
Ni/GC-PtNPs-HT [n]	0.00144	up to 8	15.0	2007	[192][246]
NANOSTRUCTURES					
Ni/Ti nano	7.32	0.05 - 0.6	1.2	2008	[3]
Ni NWA[o]	1.043	$5x10^{-4}$ – 7.0	0.1	2009	[247]
CuO-NFs-GC	0.4313	$6x10^{-3}$ - 2.5	0.8	2009	[248]
CuO nanosphere/Nafion	0.40453	up to 2.55	1.0	2008	[249]
Cu nanobelt	0.0798	0.01 – 1.13	10.0	2009	[250]
Pt - 3D DPNs [p]	0.0121	1.0 - 20	1.2	2008	[251]
PtPb NWA	0.01125	1.0 - 11	8.0	2008	[78]
C BDD nanoforest	0.0081	up to 7.0	0.2	2009	[153]
Pt nps CNFs[q]	0.00052	2.0 – 20	not given	2010	[159]
CuO nanowires	0.00049	$4x10^{-4}$ - 2.0	0.049	2008	[252]
CuO nanorod	0.00045	0.01 - 0.1	1.2	2008	[168]
UNMODIFIED SURFACES					
Ni foil	0.67	0.01 - 10	1.8	2010	[106]
C graphite pencil	not given	$5.5 x10^{-3}$ – 0.044	0.1	2009	[253]
INTERCALATED/IMPLANTED					
IrO2 in PbO2 matrix	$8.30 x10^{-9}$	0.1 - 20	50.0	1997	[193]
Cu implanted BDD	not given	1.0 - 5.0	not given	2006	[121]
Cu/SAMs [r]	not given	$3x10^{-3}$ - 10	0.7	2006	[254]
Cu-TO[s]	not given	$2.5x10^{-4}$ - 8.0	0.1	2009	[255]
CHEMICALLY MODIFIED SURFACES					
Ni powder MCC [t]	$4.00 x10^{-5}$	$5x10^{-4}$ - 5.0	0.1	2005	[216]
Ni(II)-curcumin	not given	$1x10^{-3}$ - 10	0.1	2006	[256]
Ni-Al-NO3 ldh [u]	not given	$7x10^{-4}$ -1.2	0.5	2009	[185]
NiCu/GC	not given	1.0 - 9.0	0.8	2009	[122]

List of abbreviations: a- gold film electrode, b- inverse opal, c- ordered mesoporous, d- nanocube, e- carbon nanotubes, f-quercetin, g- ionic liquid, h- nanotube array, i- carbon fiber paste, j- carbon ionic liquid electrode, k- indium tin oxide, l- three dimensional silicate network, m- exfoliated graphite, n- hydrothermal, o- nanowire array, p- dendritic platinum nanostructures, q- carbon nanofibers, r-self assembled monolayers, s- titanate, t- modified carbon ceramic, u- layered double hydroxide

This is mostly due to a lack of selectivity at the electrode, but also the slow kinetics of glucose oxidation at many 'bare' electrodes, fouling of the electrode by real sample constituents, and the limited number of systems that are applicable to physiological pH.

Table 1 lists the non-enzymatic glucose sensors that have been developed that use fixed potential amperometric analysis, as would be used in physiological application. Limits of detection are

generally within the realm of blood glucose concentration (i.e. 2-10 mM), sensitivity varies with respect to surface structure and the catalyst used, with the development of nano-dimensioned materials in the last decade coinciding with a sudden surge in non-enzymatic systems. In the medium fixed potential amperometry coupled with of flow injection analysis (FIA) and liquid chromatography (LC), non-enzymatic glucose sensors were found to be sensitive and durable electrodes, with the problems of selectivity and electrode fouling addressed by the use of chromatographic stages.

Table 2. Non-enzymatic glucose sensors that have used fixed potential, chronoamperometric methods for electrochemical determination coupled with separation stage such as liquid chromatography and flow injection analysis. The table is presented with respect to electrode type, sensitivity, linear range, limit of detection and publication year. Abbreviations are given at the foot of the table.

Electrode composition	sensitivity (mA mM^{-1})	Linear Range (mM)	LOD (μM)	Year	Reference
POROUS OR INTERCALATED LAYERS					
Cu nanoporous	220	0.01 - 0.5	40.0	2009	[257]
Cu implanted BDD	6.4 $x10^{-3}$	0.001 - 0.05	1.50	2004	[144]
Ni implanted BDD	2.8 $x10^{-3}$	0.001 - 0.05	0.67	2004	[144]
Ni–AuBPE[a]	3.12$x10^{-4}$	1$x10^{-4}$ - 2.0	0.031	2009	[258]
Ni-NDC	not given	5$x10^{-5}$ - 0.5	0.02	2003	[230]
NANO-TUBE/PARTICLE/STRUCTURE					
Cu/CNT	0.602	5$x10^{-4}$ - 1.8	0.10	2009	[131]
Bi-modified/Pt	0.35	0.0011 - 1.2	1.10	1996	[259]
Cu mps Au elect.	not given	4$x10^{-5}$ - 0.7	8.0 $x10^{-7}$	1997	[260]
Au nanofract. film	not given	up to 57.5	0.75	2006	[85]
METAL / UNMODIFIED					
Cu wire	37.9	0.01 - 1.0	5.0 $x10^{-6}$	1997	[261]
Cu electrode	2.30	0.001 - 0.05	10.0	2004	[144]
Ni electrode	1.60	0.001 - 0.05	91.0	2004	[144]
Cu RDE[b]	2.30$x10^{-3}$	not given	1.0 $x10^{-3}$	2001	[262]
Ni	not given	0.1 - 2.5	40.0	2007	[113]
Ag	not given	0.001 - 1.0	2.0 $x10^{-3}$	1991	[263]
Au	not given	0.001 - 1.0	0.03	1991	[263]
Cu	not given	1$x10^{-4}$ - 0.1	0.03	1991	[263]
Ni	not given	0.001 - 1.0	0.20	1991	[263]
Ni BPE	not given	3 orders of mag.	0.48	2008	[264]
Pt	not given	1$x10^{-4}$ - 0.1	0.20	1991	[263]
Ni wire	not given	not given	5.90 $x10^{-7}$	1990	[265]
Cu	not given	2$x10^{-10}$ - 1$x10^{-6}$	2.00 $x10^{-7}$	1996	[115]
Cu	not given	not given	5.90 $x10^{-5}$	1994	[266]
Cu/Cu(OH)2	not given	not given	2.36 $x10^{-5}$	1994	[266]
Ni	not given	not given	1.77 $x10^{-4}$	1994	[266]
Ni/Ni(OH)2	not given	not given	5.90 $x10^{-5}$	1994	[266]
Ni-MCH	not given	not given	2.50 $x10^{-5}$	1994	[267]
Ni(III) oxide	not given	not given	0.0944	1986	[268]
Au	not given	6$x10^{-4}$ - 10	0.50	1988	[269]
Au with Nafion membrane	not given	not given	0.025	1989	[194]
Pt	not given	0.02 - 0.5	20.0	2002	[270]

CHEMICALLY MODIFIED ELECTRODE					
Cu2O/CPE	0.215	not given	1.18 x10^{-6}	1991	[117]
Co/GC	0.01	5x10^{-4} - 0.5	5.31 x10^{-6}	1992	[271]
Ni-CME[c]/GC	8.60x10^{-3}	0.005 - 0.5	5.90 x10^{-6}	1995	[272]
RuOx-RuCN mv[d]	6.20x10^{-3}	0.3 - 20	40.0	2005	[273]
Ru02	6.30x10^{-4}	from 1x10^{-6}	1.60 x10^{-5}	1990	[274]
Ni tetra ruth porph	not given	0.0025 - 1.0	0.36	2005	[275]
Eastman-AQ[e]/Ni(II)	not given	0.010 - 50	5.00	1991	[276]
Cu- coated	not given	6.2x10^{-5} - 0.4	1.20 x10^{-6}	1989	[277]
Ni-CME GC	not given	0.0025 -0.1	2.50 x10^{-6}	1991	[278]
Co-modified GC	not given	not given	2.36 x10^{-4}	1994	[266]
Cu-modified GC	not given	not given	2.07 x10^{-4}	1994	[266]
Ni-modified GC	not given	not given	5.90 x10^{-5}	1994	[266]
ALLOY					
Ni-Ti alloy	2000	not given	5.00 x10^{-8}	1999	[279]
Ni-Cr-Fe	36.6	1x10^{-4} - 0.5	1.00 x10^{-6}	1993	[280]
Ni-Cr	30.0	1x10^{-4} - 1.0	5.00 x10^{-7}	1993	[228]
Ni-Cu	27.7	0.001 - 0.5	1.50 x10^{-5}	1993	[280]
Ni-Ti alloy	19.6	1x10^{-4} - 1.0	5.00 x10^{-5}	1994	[123]
NiCr	not given	0.001 - 1.0	0.033	2001	[124]
ELECTROCHEMICALLY DEPOSITED					
Cu oxide-CP	1000	1x10^{-8} - 1x10^{-6}	3.00 x10^{-6}	1994	[116]
Ni(OH)2/GC	13.80	not given	3.40	2009	[225]
Au /Cu bimetallic	0.24	4 orders of mag.	4.00 x10^{-6}	1998	[182]
Au-Ni(OH)2	0.0277	6x10^{-5} - 0.7	1.50 x10^{-6}	1999	[281]
Co/GC	not given	3x10^{-4} - 3.0	0.30	2003	[282]
Ni(CN)2 on Au	not given	6x10^{-5} - 0.2	3.30 x10^{-6}	2001	[229]
POLYMER AND COMPOSITE					
NiOx polymeric	0.27	0.1–1000	56.0	2002	[283]
Cu2O-CCE	2.56x10^{-3}	up to 5.0	8.00 x10^{-5}	1997	[284]
PVdF/PAPBA[f]-NFM [g]	2.00x10^{-3}	0.1 - 16	100	2007	[285]
Au /Nafion	2.34x10^{-4}	5.0 - 60	1200	2001	[286]
Cu/p-1-NAP[h]/GC	not given	0.001 - 0.5	5.00 x10^{-5}	2003	[287]
NiO-CP	non given	0.001 - 1.0	0.30	2007	[288]
PEG/Cu2O CPME	not given	0.001– 0.05	0.12	2007	[226]
Cu ions/p-1-NAP	not given	0.001 - 0.3	8.00 x10^{-5}	1999	[227]

List of abbreviations. a- barrel plated electrode, b-rotating disk electrode, c- chemically modified electrode, d-multivalent, e-anthraquinone, f- poly(vinylidene fluoride) poly(aminophenylboronic acid), g- nanofiborous membrane, h- poly-1-napthylamine

These are shown in Table 2, and it can be seen that the limits of detection are considerably lower, and the electrodes are generally less elaborate. Table 3 lists the non-enzymatic electrodes that have used variable potential amperometric methods in their analytical approach such as potential sweep and pulsed voltammetry, therefore again losing the imposed selectivity and increased sensitivity of the FIA and LC.

Direct non-enzymatic electrooxidation of glucose varies considerably depending on the electrode material used. Electrocatalytic processes are essential to glucose oxidation, as it is otherwise

a kinetically very slow process that would produce no discernable faradaic current at most commercial electrodes. This explains why direct glucose oxidation often does not adhere to scan rate dependent voltammetry, indicating a non-diffusion controlled process [4,38]. A number of electrode materials have been used over the past 60 years; some are more frequently used than others. The following sections discuss the electrooxidation process for glucose at a number of electrodes, namely platinum, gold, nickel, copper and carbon electrodes.

3.1.1. The mutarotation of glucose

Glucose has three aqueous isomers denoted α-glucose (α-G), β-glucose (β-G) and γ-glucose (γ-G) shown in Figure 2.

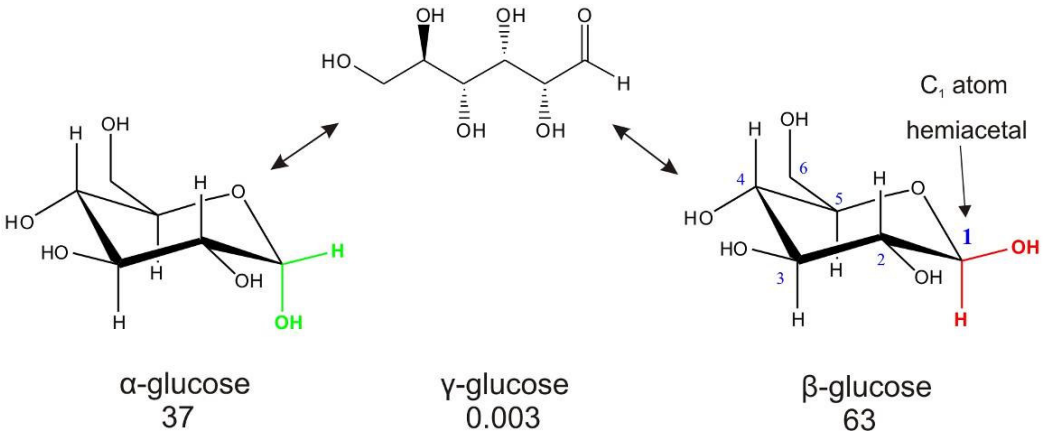


Figure 2. The interconversion of glucose anomers (α, β and γ) and their relative ratio at pH 7. The hemiacetalic C_1 atom is highlighted to indicate the difference between the two anomers.

The former two species are the most abundant in solution, and correspond to cyclic hemiacetal forms. γ-G is an intermediate between the two cyclic groups, and corresponds to a linear, free-aldehyde form of the compound. When at equilibrium in water at room temperature these isomers are present in the ratio of 37:63:0.003 for α, β and γ respectively [39], thus indicating that glucose is most stable in its cyclic form.

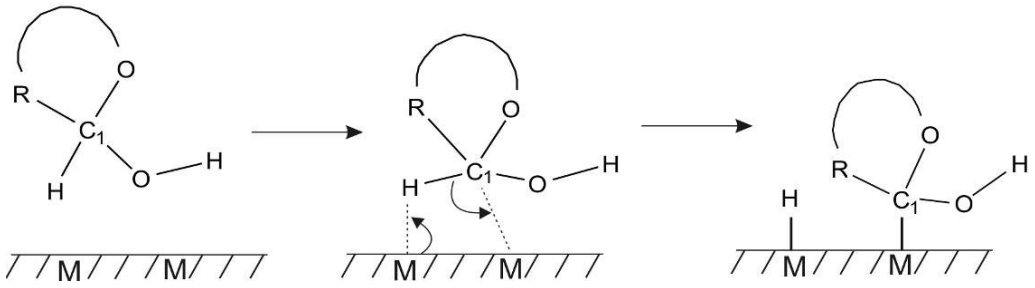
The enzymes GOx and GDH are anomer specific, responding only to β-G and as such an awareness of the ratio of α to β glucose in the blood is necessary. Glucose interconverts between the α and β form until a thermodynamic equilibrium is established. In water at pH 6.8 this can take over 2 hours at room temperature (ca. 24ºC) [40], and blood is found to have approximately the same ratio of glucose anomers as in water [41]. Mutarotation follows first order kinetics, the rate of interconversion increasing with temperature, yet the process is very slow, and even at temperatures of 45 ºC the ratio of α to β only shifts to ca 40:60 after equilibrating. As the cells uptake β-G in the blood for use in glycolysis, the equilibrium is actually maintained by mutarotases [42], enzymes secreted at the kidneys and liver that accelerate the interconversion of α to β glucose. As such, changes in temperature when

conducting in-vitro analysis of blood is not a concern. The rate of mutaroation is acid base catalysed however, with alkaline solution shown to favour β-G in a 20:80 ratio of the anomers [39]. As such, the pH of a solution is highly important when conducting glucose tests that are to reflect the whole blood glucose concentration, as the physiological ratio of α to β glucose can shift significantly. Furthermore, an anomeric effect has been observed for glucose in which β-G is oxidised at a faster rate than α-G, suggesting a preferred orientation for glucose adsorption on platinum electrodes [39,43].

3.1.2. Theories of non-enzymatic electrocatalysis

Non-enzymatic electrocatalysts come in a number of forms, specifically; metals (e.g. Pt, Au), metal oxides/ semiconductors (e.g. $Ni(OH)_2$, $RuO_{2,}$), alloys (e.g. PtPb, PtRu), complexes (e.g. cobalt phthalocyanine) and carbon based (e.g. carbon nanotubes, boron doped diamond). Apart from the latter all the catalysts listed are dependent on a transition metal centre. The process of electrocatalysis is generally observed to occur via the adsorption of the analyte to the electrode surface, a process that presumably involves the d-electrons and d-orbitals of the metal substrate that allows it to form a suitable bond with the adsorbate [44]. This bond is required to form and break in the midst of the catalytic process, and as such a bond of intermediate strength would be ideal, so as not to limit adsorption or hinder desorption at any stage. The bond strength is dependent on the Gibbs energy of adsorption. Alternatively a change in bond strength may be imposed by a change in the metal oxidation state, which might alter the adsorbate-metal interaction and encourage desorption of the product [44].

The geometry of the electrode is also highly important, as is evident in numerous single crystal studies of glucose oxidation [45,46] and other organic molecules [44]. Pletcher suggested the catalytic process may occur via a concerted step, i.e. the process of hydrogen abstraction occurs simultaneously to the adsorption of the organic species. Indeed, the rate determining step in most glucose electrooxidation experiments is deemed the removal of the hemiacetalic hydrogen atom [47] (see Figure 2), and the chemisorption of the analyte is generally considered to occur simultaneously. This would mean that adjacent metal active sites would be occupied by a single adsorbate at any time, in accordance with Scheme 1.

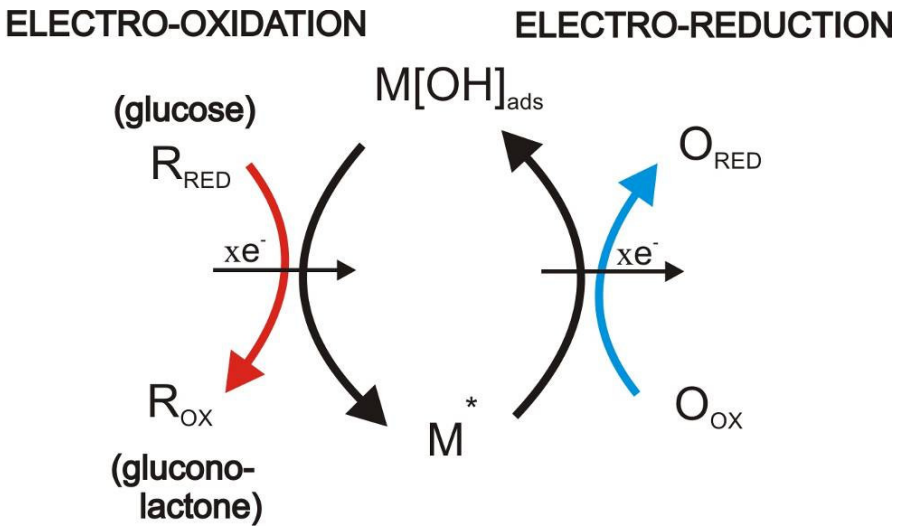


Scheme 1. An illustration of the concentric adsorption theory with adjacent adsorption sites proposed by Pletcher [44].

As such, in the fabrication and study of electrocatalysts both electronic and geometric factors must be considered, so as to fully exploit the kinetic enhancements offered by well spaced adsorption sites and increased surface area.

The proposal of active transition metal centres across the electrode only explains the adsorption process onto the surface however, but fails to consider the oxidative role of hydroxyl radicals. It has been evident in numerous publications; [46--50] that electrooxidation of glucose and many other organic molecules coincides with the onset of adsorbed OH_{ads} . Burke [51] discussed the importance of this hydrous oxide layer on the electrocatalytic process, and proposed the 'Incipient Hydrous Oxide Adatom Mediator' model (IHOAM). This was based on the observation that 'active' surface metals atoms undergo a premonolayer oxidation step that forms an incipient hydrous oxide layer of reactive OH_{ads} that mediate oxidation and inhibit reduction of kinetically slow electrode reactions. The active sites of the electrodes surface are considered to have a low lattice co-ordination value (LCN), and as such lack normal lattice stabilization energy. This occurs frequently across a polycrystalline surface at discontinuous areas such as grain boundaries and edges, and also across the surface generally due to direct exposure to the solution relative to the bulk crystal structure. Due to a lack of stability they are more reactive, and thus undergo premonolayer oxidation at lower potentials than thermodynamic surface oxidation predicts.

The catalytic importance of the active OH_{ads} layer was well known with respect to small organic compound oxidation, as the formation of the hydrous species was recognised as a fast, pre-oxidation step following chemisorption of the glucose molecule [38,43,48,52]. The hydrous premonolayer then mediates oxidation of the adsorbed species at potentials considerably lower to normal. This process is illustrated in Scheme 2.



Scheme 2. A schematic illustration of the IHOAM model in which M^* is the reductive metal adsorption site, and $M[OH]_{ads}$ is the oxidative adsorbed hydroxide radical. The scheme shows how both oxidative and reductive processes are catalysed at the metal surface.

Both the activated chemisorption model [44] and the IHOAM model [51] will be considered in the following sections regarding each main metal electrode individually. It seems that the IHOAM model is most apt for enhanced catalysis of the platinum group metals (i.e. platinum, ruthenium, iridium and palladium) and gold. This is possibly due to the majority of research regarding non-enzymatic electrocatalysis involving these metals however. The hydroxyl group undoubtedly plays a role in the electrocatalysis of glucose at nickel and copper electrodes too, as seen later on, however in these cases an incipient hydrous premonolayer is not formed to induce catalysis, but an oxidation state change of the metal hydroxide.

Table 3. Non-enzymatic glucose sensors that have used sweeping potential methods such as linear sweep, cyclic and square wave voltammetry for electrochemical determination. The table is presented with respect to electrode type, sensitivity, linear range, limit of detection and publication year. Abbreviations are given at the foot of the table

Electrode composition	sensitivity (mA mM^{-1} cm^{-2})	Linear Range (mM)	LOD (mM)	Year	Reference
POROUS/NANOTUBE/NANOPARTICLE/NANOSTRUCTURED					
Au nps on thin Au	0.16	0.5 - 8	0.50	2006	[88]
Au nano/Ti	0.14	1.0 - 15	0.0148	2009	[84]
Au nanowire array	0.04	0.1– 20	0.003	2009	[93]
Au nps on NH2-HSM[a]	0.0025 *	0.2 - 70	0.10	2007	[89]
Au-chitosan nps	not given	0.4 - 10.7	0.37	2009	[90]
BOx[b] nps MWCNTs	not given	1.5 $x10^{-3}$ - 0.26	8.00 $x10^{-4}$	2009	[289]
PdNPs func-CNTs	not given	5.0 $x10^{-4}$ - 0.04	9.00 $x10^{-5}$	2009	[290]
Au nps DHP[c] film/GC	not given	0.09 - 9.9	0.01	2007	[91]
METAL/UNMODIFIED					
C BDND[d]	0.1525	0.25 - 12	0.025	2009	[155]
C BDD micro & nano	0.1019	0.25 -10	25.0	2009	[151]
Ni DLC[e]	not given	1 $x10^{-3}$ - 0.45	not given	2009	[291]
BDD	not given	0.5 - 10	0.50	2005	[152]
Ni-BDD	not given	not given	not given	2009	[150]
ELECTROCHEMICAL MODIFICATION					
Au-chitosan nps	not given	0.4 - 10.7	0.370	2009	[90]
Ni/1-NAP [f]	not given	0.04–1.0	6.00 $x10^{-3}$	2004	[213]
NiOx PANI [g]	not given	1.0 - 100	not given	2007	[292]
POLYMER AND COMPOSITE/OTHER					
MIP Au	16.5 *	5 $x10^{-3}$ - 0.12	2.0 $x10^{-6}$	2009	[293]
Au-chitosan nps	not given	0.4 - 10.7	0.37	2009	[90]
Ni/1-NAP	not given	0.04 – 1.0	6.00 $x10^{-3}$	2004	[213]
CoPcTS [h]	not given	0.25 - 20	0.10	2008	[294]
MIP[i] Au/o-PDA [j]	not given	0.1 - 20	0.05	2001	[295]
Au nps DHP film/GC	not given	0.09 - 9.9	0.01	2007	[91]

List of abbreviations: a- amine functioned hexagonal mesoporous SiO_2, b- boron oxide, c- dihexadecyl hydrogen phosphate, d- boron doped nano diamond, e- diamond-like carbon, f- poly-1-napthylamine, g- polyanaline, h- cobalt (II) phthalocyanine tetrasulfonate, i- molecularly imprinted, j- phenylendiamine

* - these values are in units of mA mM^{-1}

3.1.3. Nanomaterials in glucose sensors

Recent advances in the development of glucose sensors, enzymatic and non-enzymatic, revolve around the increase in the synthesis of nanomaterials.

Nanoporous and microporous materials have raised particular interest, as is evident in Tables 1, 2 and 3, for non-enzymatic glucose sensors alone. Porous materials offer surfaces with a high surface to volume ratio. As such they have made an impact on several industries such as microelectronics, clean energy, environment, petroleum and gas, manufacturing and agriculture. A selection of nanostructured materials that have been used in non-enzymatic glucose sensing are shown in Figure 3.

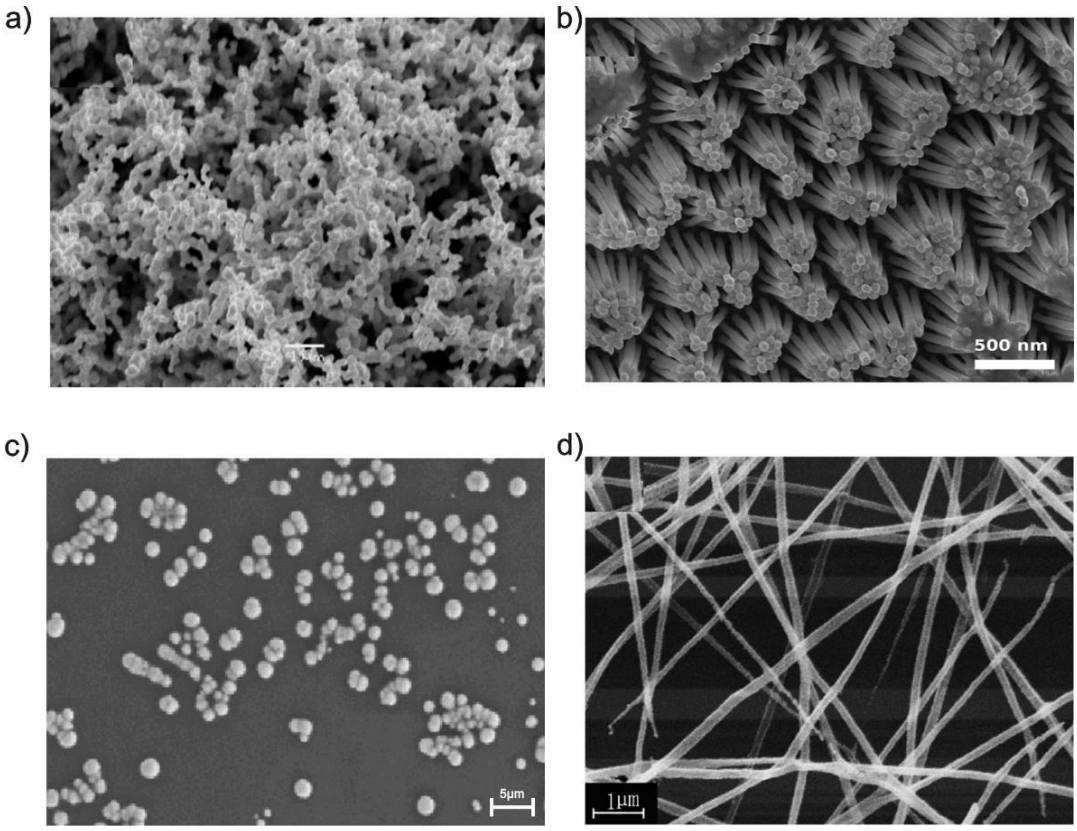


Figure 3. A selection of nanostructured nonenzymatic electrode materials. a) PtPb nanoporous (from [77]), b)Au nanowire array (from [93]), c) Ni microparticles on BDD(from [106]) and d) CuO nanofibers (from [248]). Figures reproduced with permission from all cited references.

For platinum and gold electrode the activity of the electrode is often considered in terms of its roughness factor, in which the greater the surface roughness, the greater the electrochemical activity. This is directly related to the need for active sites for adsorption as discussed by Pletcher [44] previously. Nanoscopic electrode materials impose a very high active surface area that is significantly greater than the geometric surface area, which is ideal for a kinetically controlled, surface bound reaction such as glucose oxidation. The active surface area of a porous platinum electrode has been

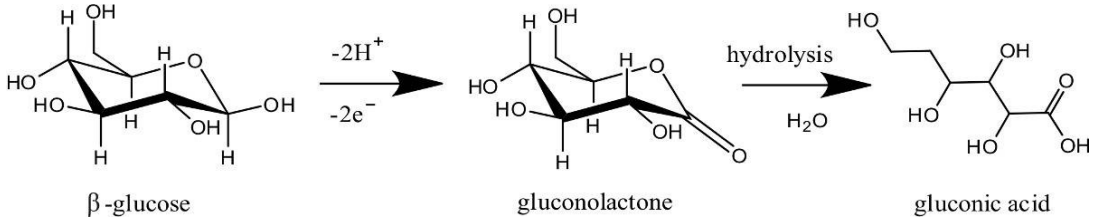
shown to be up to 600 times greater than a planar polycrystalline platinum electrode [53], and as such kinetically diffusive interference reactions are negligible relative to the adsorptive reactions.

The introduction of carbon nanotubes to enhance electrocatalysis also demonstrates the advantages posed by increasing surface area. Due to the intrinsic electronic properties of carbon nanotubes, modifying a planar electrode with the material produces a carbon electrode surface that is essentially porous, offers a very high surface area, and is very diverse with respect to modification and range of application. The use of surface modified carbon nanotubes is significant in the literature, as can be seen in Table 1 in which 20 electroanalytical reports of glucose electrooxidation using carbon nanotubes are reported. Here we see carbon nanotubes decorated with nanoparticles of platinum [54,55], bimetallic platinum [55--57], copper [58,59] and nickel [60], all reporting a doubly enhanced response due to the carbon nanotubes and metal nanoparticles.

3.2. Platinum electrodes

3.2.1. Mechanism of glucose oxidation at platinum

A large amount of the work first investigating the direct electrooxidation of glucose was performed at platinum electrodes [38,43,48,52,61--64]. Researchers have explored the behaviour of glucose at a platinum electrode in acid [61], neutral [38,52,62,63] and alkali [38] conditions. A common conclusion from a number of authors was that the sole product of oxidation is gluco-δ-lactone, which hydrolyses to gluconic acid on standing, regardless of the solution pH (Scheme 3) [38,48].



Scheme 3. The $2e^-$ oxidation of glucose to gluconolactone and further hydrolysis to gluconic acid

However, spectrochemical evidence regarding intermediate adsorbates has frequently disagreed, suggesting reduced CO_2, CO_{ads} and fragments of the glucose molecule to also be present as oxidation products [43,63].

The cyclic voltammetry of glucose at a platinum electrode is reflective of the three distinct areas associated with platinum voltammetry, though it may vary significantly depending on electrolyte and temperature conditions. Investigation by Vasil'ev et al [38,48] and numerous other authors [43,46,52,61--64] all conclude that in the anodic sweep three oxidation peaks are observed. A typical cyclic voltammogram of glucose on a platinum electrode at pH 7 is shown in Figure 4 (edited from ref [64]) with each peak numbered, including the two cathodic oxidation peaks 4 and 5. Platinum and

platinum group metals electrocatalyse glucose oxidation in a manner that is, for the most part, analogous to the electro oxidation of methanol, methanal and methanoic acid [38,48,49,65,66]. Scheme 4 illustrates the 3 oxidation mechanisms associated with each potential region of the platinum electrode. These are as follows.

Peak 1 – The chemisorption and dehydrogenation of glucose in the *hydrogen region.* This initial step is the dehydrogenation of the glucose molecule at the hemiacetalic carbon 1 atom (C_1) [43], and adsorption of the glucose molecule onto the platinum surface (Sch. 4a). This occurs in the potential region of > 0.3 V vs. RHE, with the removal of the first hydrogen atom considered the rate determining step [38,47].

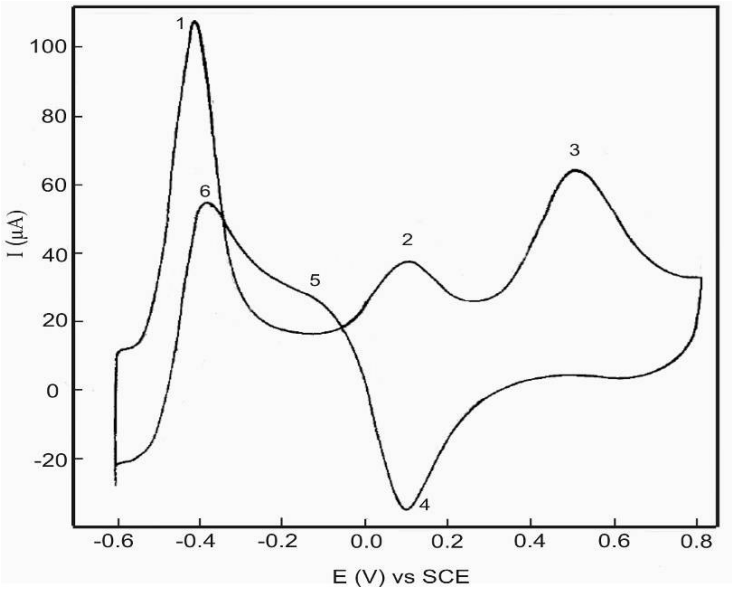


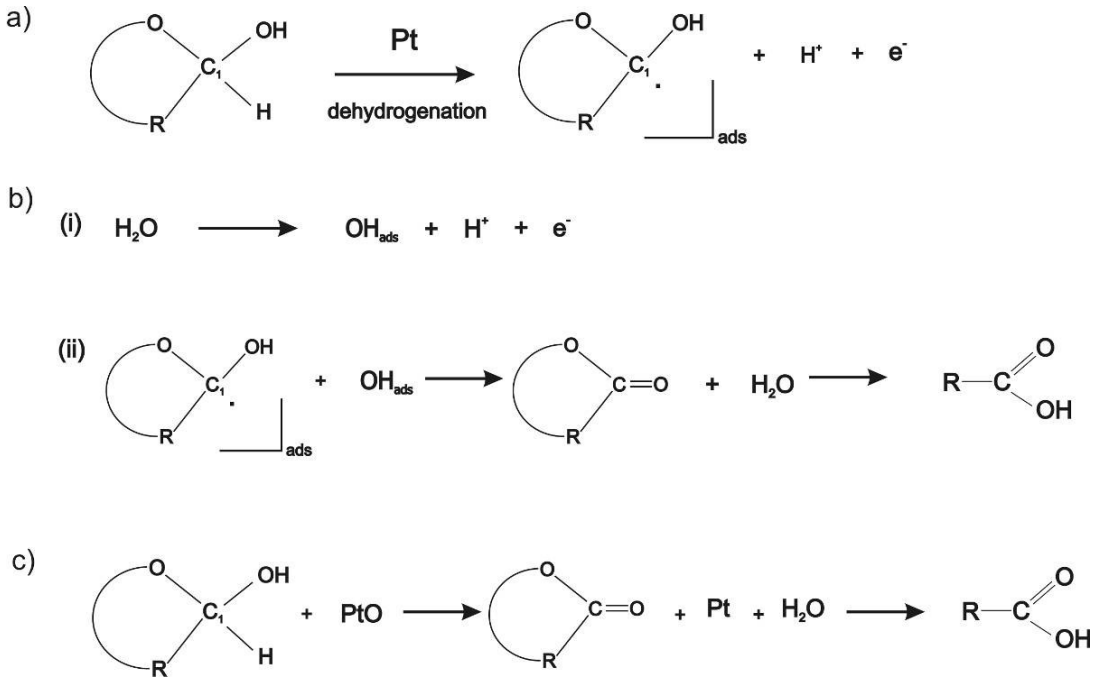
Figure 4. Typical cyclic voltammogram of glucose oxidation (0.1M) taken at 30°C on a bright platinum electrode in pH 7 phosphate buffer (0.2 M). Edited from Figure 7 in reference [64] Reproduced with permission from [64]. Copyright 2010, The Electrochemical Society

Peak 2 – Electrooxidation of the chemisorbed species occurs in the *double layer region* spanning <0.3 to <0.6 V vs. RHE. At increasingly anodic potentials, the abundance of OH_{ads} species increases, as the fast dissociation of water reaction (b(i)), and subsequent adsorption of the hydroxide anion occurs. In accordance to the IHOAM model, the incipient hydroxide is catalytic to the oxidation of the adsorbed glucose, thus accelerates electro oxidation by following reaction b(ii). Thus the maxima for Peak 2, and surface bound glucose oxidation is observed at the potential onset for pre monolayer oxygen adsorption on platinum [38,48,51,52].

Peak 3 – The *oxygen region* from ca. >0.7 V vs. RHE to the onset of bulk oxygen evolution. Here the platinum surface is covered by a monolayer of adsorbed oxygen, initially inhibiting glucose oxidation as OH_{ads} is desorbed and replaced by less catalytically active O_{ads}. However, as soon as a suitable PtO film has formed, direct catalytic oxidation of the bulk glucose solution may occur. This is

observed experimentally as the reaction kinetics become diffusion controlled rather than surface bound [61,62], and inhibition of the reaction is now observed as other oxy-compounds are formed at the platinum instead of platinum oxide. This reaction is shown in Scheme 4c, and is followed by the immediate regeneration of the PtO layer.

In the above approach it is assumed that the glucose oxidation pathway follows Scheme 3, i.e. glucose to gluconolactone to gluconic acid. However spectrochemical evidence of glucose oxidation suggests otherwise. Bolzan et al [63] used online mass spectrometry to explore the oxidation intermediates and products observed during glucose oxidation on platinum.



Scheme 4. A possible mechanism for the oxidation of glucose at a platinum electrode.
a) Glucose is adsorbed onto the platinum surface following hydrogen abstraction at the C_1 position. This dehydrogenation process is observed in peak 1 of Figure 4.
b) (i) the dissociation of water to produce hydroxide anions
(ii) the subsequent oxidation of adsorbed glucose by the adsorbed hydroxide ions
c) Oxidation of glucose by PtO in the oxygen region of the scan in Figure 4, peak 3

They found that CO_2 was formed in the hydrogen region via the hemiacetalic C atom, via decarboxylation of a gluconate intermediate. In the double layer region a number of strongly adsorbed intermediates are formed bound at the C1 and other C atoms which then oxidise to CO_2. Beden et al [43] used Fourier transform infrared reflectance spectroscopy (FTIRS) to discern the structures of the adsorbed intermediates. They claimed that in the double layer region the dehydrogenated glucose species was adsorbed as the gluconate bound at one or two oxygen atoms, but at E< 0.6 V the intermediate was adsorbed gluconolactone. The latter investigation again stressed the importance of 'active' OH^- anions in the vicinity of electrode surface to drive the double layer oxidation by breaking

the C-O-C bond. However, Beden makes no claim to have observed the formation of CO_2 at the surface.

Skou [61] investigated the oxidation of glucose in the PtO region (i.e. Peak 3) in both acidic and neutral conditions. It was observed that relative to the blank solution, surface oxide formation was inhibited in the region of Peak 2, presumably due to the presence of adsorbed glucose, yet a large oxidation peak (Peak 3) was observed at higher anodic potentials. The peak current of Peak 2 was found not to alter with increasing scan rate, yet Peak 3 was scan rate dependent.

3.2.2. Disadvantages of platinum electrodes

The oxidation of glucose on platinum strongly depends on the electrolyte conditions, particularly the nature and the concentration of the ions present [64]. This is due to the dependence of glucose adsorption on the availability of the platinum surface. Competitive adsorption by other anions, especially true of phosphate anions [38,64], the extent of hydrogen and hydroxide adsorption, and the isomeric structure of the glucose molecule (i.e. α, β or γ glucose) [39] all influence the extent of glucose chemisorption, and therefore the extent of glucose oxidation. The HPO_4^{2-} anion (pKa 12.48 at 25ºC) is the most abundant at pH 7.7 to pH 12.03 and is also has the greatest affinity for adsorption onto platinum out of the phosphate anions. It is therefore necessary to consider the competitive adsorption of the anion when investigating glucose electrooxidation on platinum in neutral phosphate buffers that do not reflect human blood conditions [64,67].

Because of the dependence of glucose oxidation on the degree of adsorption to the electrode surface, direct proportionality between the oxidation current and glucose concentration is lost as soon as the electrode surface is saturated. This is a limiting factor for platinum electrodes, as the linear range for glucose oxidation becomes dependent on the electrode surface area, and this is over the physiological glucose concentration range of 2 to 30 mM. Furthermore, the activity of the platinum electrode largely dictates the extent of the catalytic current for glucose, and is difficult to reproduce from one experiment to the next [38]. The electrode surface structure is paramount to the electroactivity of platinum towards glucose oxidation, and a number of other small organic molecules. Adsorption sites vary across various single crystal and polycrystalline surfaces thus greatly altering adsorption and surface activity, and therefore the fundamental kinetics of glucose oxidation at platinum. The onset and degree of catalytic activity therefore varies depending on surface structure, as researchers have observed [44,46,48].

One of the biggest drawbacks of using platinum electrodes however, especially in the physiological condition, is its tendency to undergo poisoning from so many species. Within physiological solutions numerous species exist that immediately inhibit the electroactivity of platinum. One of the most significant constituents is chloride anions, which strongly chemisorb to the surface of platinum and thus render the surface inaccessible to glucose, hydrogen, and hydrous oxide, particularly in acidic solutions [38]. Other organic compounds also severely reduce the ability of platinum, in particular amino acids and other blood based proteins, and electroactive compounds such as uric acid (UA), ascorbic acid (AA) and acetaminophen (AP), which also strongly adhere to the surface and

react. However, even in the absence of the extraneous species, platinum can undergo self poisoning during the oxidation of glucose, simply due to the adsorption of the oxidation products and intermediates [43,63]. Essentially the surface of a platinum electrode is non selective to what adsorbs onto it, and as such, poorly diffusive molecules such as glucose, can not compete for electroactive surface over any length of time.

Finally, the significant cost of platinum far outweighs its practical use in disposable glucose sensors, and without a doubt their application would vastly increase the cost of the overall product. This is a factor that can not be ignored in the search for practical, non-enzymatic glucose sensors, as the prevalence of the disease rapidly increases in economically poor areas of the world, and the enzymatic alternatives are screen printed carbon based electrodes.

3.2.3. Recent advances in platinum electrode fabrication

From Table 1 it is evident that despite the drawbacks associated with platinum electrode discussed above, the electrode material has been persistently used, and in recent years, quite successfully. The introduction of nano dimensions to electrode materials seems key to their successful application, with nanoporous [53,68--72], nanoparticle [73,74] and other nanostructured [55,75] platinum, electrodes emerging. The latter publications report enhanced amperometric detection that avoids electroactive interference due to the increased roughness factor of the electrode surface, as discussed in section 3.1.2.

A platinum iridium (Pt-Ir) nanoporous material was found to give a very strong and sensitive response to the amperometric oxidation of glucose [76], despite the presence of the electroactive interferences AA, UA and AP, and also a high concentration of chloride anion. This is in part due to the cathodic shift in optimum amperometric current to 0.1 V vs. Ag/AgCl, a potential at which the interferences are not active. The shift is attributed to the enhanced catalysis imposed by the iridium. Another platinum bimetallic porous electrode reported by Wang et al [77] reports improved sensitivity and reduced poisoning effect from Cl^-, as well as a much lower amperometric potential of -0.08 V vs. Ag/AgCl. A comparable sensitivity from a PtPb nanowire array was also reported in 2008 [78] by operating effectively at a negative potential of -0.2 V vs. SCE, again reducing the effect of unwanted electroactive interference species. Platinum nanotubule arrays [75] were reported prior to this in 2005, and shown to discriminatively enhance the catalytic response to glucose, though the sensitivity was much less than has been observed at porous platinum electrodes.

Overall, the introduction of nano materials to electrode fabrication has led to an improvement in the development of platinum electrodes, particularly with respect to a resistance to fouling and the effect of interference species.

3.3. Gold electrodes

3.3.1. Mechanism of glucose oxidation on gold electrodes

Gold electrodes are very weak chemisorbers due to filled d orbitals, yet display a higher electroactivity towards glucose oxidation than platinum, and have therefore also been greatly

researched as non-enzymatic glucose sensors. The electrocatalytic behaviour of gold is highly complex however, and despite numerous investigations, the exact mechanism for glucose oxidation at gold remains undetermined, though a variety of mechanisms have been proposed [47,48,50,51,79,80]. Vasil'ev et al [48] claimed the process to be the same as on platinum electrodes in which the C_1 hydrogen atom was first detached, and adsorbed OH species then further oxidised the adsorbed intermediate to form gluconic acid. A number of researchers disagree with this however [47,50,63,79] due to the different pH dependence, position of anodic peaks, the potential onset of glucose oxidation, and the much reduced level of self-poisoning and adsorbate poisoning on gold.

The catalytic component of gold electrode is believed to be hydrous gold oxide, AuOH, which is formed by the chemisorption of hydroxide anions to the gold surface. This effect is more pronounced at higher pHs, and occurs in the region of premonolayer oxidation of the gold surface [48,51]. Anodic sweeping voltammetry over a range of pHs found that at pH < 3 glucose could not undergo oxidation until the gold surface was suitably oxidised. At pH>4 however, the onset of glucose oxygen shifts cathodically, and is observed on the non-oxidised surface. Thus Vasil'ev et al [48] concluded that chemisorbed OH_{ads} particles take part in the slow step of glucose oxidation, explaining the pH dependence of the catalysis. This is also supported by the IHOAM model [51] of electrocatalysis, in which Burke stipulates that the electrocatalytic nature of gold is due to the hydroxide premonolayer formation.

$$H_2O \longrightarrow OH^- + H^+ \quad (3)$$

$$Au + OH^- \longrightarrow AuOH_{ads} \quad (4)$$

This process occurs at potentials of ca. -0.1 to 0.3 V vs. SCE depending on the surface structure of the gold electrode. Unlike platinum however, a chemisorption process is not evident, and therefore possibly occurs at the hydroxide surface, and is followed by rapid oxidation of adsorbed glucose by the adsorbed hydroxide anions. Investigations using fructose and sodium gluconate, both lacking the labile C_1 hemiacetalic hydrogen, have found that their oxidation is inhibited at a Au(100) surface in pH 7.4 in the double-layer potential region (i.e-0.6 to 0.6 V vs. SCE) [45,47,65]. The latter investigation by Hsiao et al also observed the deuterium labelled glucose versus normal glucose, and confirmed that the removal of the hemiacetal hydrogen was in fact the rate determining step of the reaction, as was previously observed for platinum. This strongly suggests that the hemiacetalic hydrogen is essential to the initial glucose oxidation on gold, and an adsorbed radical intermediate is observed prior to glucose oxidation [47,48].

FTIR and H_1NMR investigations showed gluconic acid, via gluconolactone, to be the sole product at potentials cathodic of the gold oxidation to higher oxides (i.e. Au_2O_3). It is possible that further oxidation of glucose occurs at these highly anodic potentials, as CO_2 has been observed as a product suggesting C-C bond cleavage [63]. Also the response of sodium gluconate on single crystal Au(100) gives two strong oxidation peaks rather than the three observed for glucose, indicating the

most cathodic peak to be the formation of the gluconic acid [45,47], or at least due to hemiacetalic carbon.

Rotating disk experiments at a polycrystalline gold electrode [47] showed a curious decrease in peak current with increasing rotation rate suggesting competitive adsorption of intermediates with impurities and the further oxidation of the adsorbed intermediates on gold electrode, which gave rise to the high oxidation currents. Larew et al [50] indeed believed that oxidation above gluconolactone occurs at gold electrodes depending on glucose concentration and electrode potential. They proposed four processes regarding the latter two parameters. First, at high glucose concentrations a 2 electron step oxidation to gluconic acid occurs. Second, gluconic acid is the product of a low glucose concentration but at a potential lower than -0.35 V. The third observation was at low glucose concentrations but potentials greater than -0.35 V, the oxidation involved more than 2 electrons. Here it was postulated that an oxidative cleavage between carbon atoms 1 and 2 may occur, as well as the oxidation of carbon atom 6. Finally, the fourth observation, to satisfy an even greater oxidative current, was the possible formation of an endiol intermediate. However, mass spectrometry investigations indicate CO_2 is not formed until the onset of higher gold oxides [63] therefore oxidation is unlikely to proceed to C-C bond cleavage within the double-layer region.

As with platinum, the mechanism of glucose oxidation on gold also depends on the gold surface structure. Significant differences in voltammetry have been observed on single crystal gold electrodes of varying crystallographic planes, and these in turn differ greatly to the process observed on polycrystalline gold [45,47,48]. The dependence on surface structure indicates a dependence on adsorption sites and surface activity, and therefore supports the activated chemisorption model [44]. On the other hand, gold is a poor chemisorber, and as such it would be deemed a very poor catalyst with respect to the latter model. Recently Burke proposed an alternative view of gold electrode surface, suggesting that its electrocatalytic behaviour arises from metastable surface states (MMS) [79]. These metastable states function as electrocatalytic redox mediators, and allow the oxidation of the surface by an active hydrous layer at a much reduced potential relative to usual monolayer oxide. This is once again the basis of the IHOAM model, but it further illustrates the dependence of gold electrocatalysis on the activity of the electrode surface and thus provides some explanation for the difference in single crystal catalysis, and the recent enhancements by nanomaterials.

3.3.2. Disadvantages of gold electrodes

Pure gold electrodes are more selective than platinum electrodes, yet still exhibit an affinity for the adsorption of chloride ions in neutral conditions [48]. It was observed that in neutral phosphate buffer solutions, glucose electrooxidation rate decreases proportionally to the chloride ion concentration, with the strongest effect observed at less positive potentials. This is believed due to the displacement of adsorbed chloride ions by adsorbed oxygen at higher potentials on the gold surface [48]. Despite a significant decrease in sensitivity to glucose when in the presence of chloride anions, the gold electrode maintains a directly proportional signal to glucose concentration over a broad linear range [48]. Alkaline conditions practically eliminate the effect of chloride, even at physiological

levels of the anion. The presence of various anions were found to be inhibitory to glucose oxidation on gold electrodes in acidic media, in particular strongly adsorbed anions such as chlorides, sulfides and phosphates [80]. The investigation found that the acidic media did not inhibit glucose oxidation, and that in the absence of the adsorbing interfering species glucose oxidation readily occurs on gold in acid. Amino acids, however, almost completely inhibit the gold electrode surface, preventing electrooxidation in both neutral and alkaline conditions, and as such a semi-permeable membrane across the electrode surface would need to be employed to protect against fouling.

Again, the cost effectiveness of using gold materials in the fabrication of disposable electrodes must be considered. As with platinum electrodes, the high cost involved is a significant disadvantage to using gold or gold modified electrodes in this application. Gold electrodes therefore remain as unsuitable to glucose biosensor applications as platinum electrodes, unless used in suitably small quantity as is possible in the fabrication of nanomaterials.

3.2.3. Recent advances in gold electrodes

Similarly to platinum, advances in gold electrodes have tended towards the use of nanomaterials in recent years. Porous and nanofractured gold surfaces [81--85], gold nanoparticles [86--91] and gold nanotubes and nanowires [92,93] have all been utilised in the past 5 years to increase the active surface area of the electrode and enhance electro-oxidation.

Bai et al [81] fabricated a unique electrode material described as a three dimensional gold film electrode (3DGFE). The electrode was found to be highly catalytic towards glucose relative to other gold porous electrodes, and was able to operate effectively at a negative potential of -0.3 V vs. SCE in a NaCl electrolyte. As such the electrode observed no interference from AA, UA or AP, and also proved to be unaffected by the presence of chloride anions in the solution. A similar response was obtained by a highly porous polycrystalline gold electrode treated by an amalgamation process [82]. Again the operating potential for glucose detection could occur at a more negative potential of -0.1 V vs. SCE, thus avoiding electroactive interference species.

Gold nanoparticles seem to have had the greatest impact on enhancing glucose electro-oxidation currents, as we observe almost a ten fold increase in sensitivity relative to porous electrodes (see Tables 1 and 3). Traditionally, gold nanoparticles are synthesised chemically with stabilisers such as thiol or chitosan so help retain the particulate structure. However, the use of electrochemical approaches [94,95], or seed mediated growth techniques allow the fabrication of pure gold particles without the catalytically hindering effect of the stabilising molecules. In 2009 Ma et al [86] electrochemically fabricated a gold nanoparticle (Au np) modified indium tin oxide (ITO) electrode, forming a gold nanoparticle array ranging from 20 to 60 nm in diameter. The Au np ITO electrodes were applied to nonenzymatic glucose sensing, and observed a high sensitivity of 183.5μA mM^{-1}, though the linear range did not encompass the physiological level of glucose. A high response to glucose was also observed from a sol-gel fabricated Au np modified silicon network electrode [87] in which a sensitivity of 0.179 nA cm^{-2} nM^{-1} was achieved. In the latter work, the sensitivity of glucose catalysis on gold with respect to particle size and surface orientation was again demonstrated, as the

greater variety of crystal planes in the enlarged gold nanoparticles encouraged earlier onset of glucose oxidation, and improved catalytic activity at a lower potential [87]. These results mirror the metastable surface states theory by Burke [51,79], indicating that a higher degree of surface active sites such as grain boundaries and crystal defects, result in the earlier onset of surface oxidation, thus mediating electrocatalysis at a significantly lower potential.

A further improvement to the catalytic effect of gold was found by the addition of ad-atoms to the gold surface. Metals including Ag [96], Hg, Cd, Cu and platinum group metals Ru, Pd, Ir and Pt [97] have all been deposited on gold surfaces, with silver and mercury only displaying catalytic improvement. Silver underpotential deposition (UPD), forming a 1/3 monolayer across the gold surface was found to greatly improve catalysis, shifting the oxidative response of single crystal gold ca. 0.2 V to more cathodic potentials [96,97]. Such a pronounced negative shift can have significant benefits with regards to avoiding interference effects, as has been observed on bimetallic platinum electrodes [76--78].

3.4. Nickel electrodes

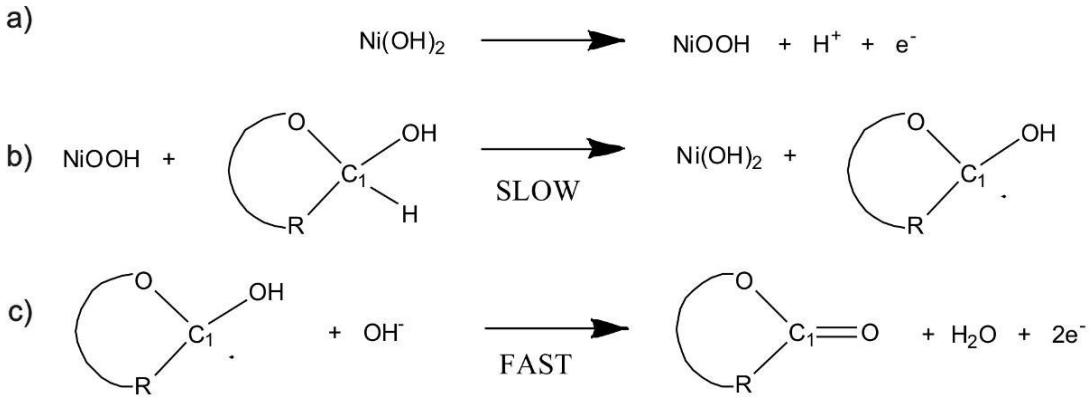
3.4.1. Mechanism of glucose oxidation on nickel electrodes

Nickel electrodes have been extensively explored as catalysts of organic compound oxidation in an alkaline medium. Fleishmann at al [98] published a thorough investigation into the behaviour of a nickel anode on the electrooxidation of numerous organic materials, including glucose. It was established, in agreement with a number of other works [99--101], that the catalytic component is a Ni(III) oxyhydroxide species, the oxidized partner to the $Ni(OH)_2$/NiOOH redox couple. This is a surface bound change of the nickel oxidation state, and may be simply represented by the equation:

$$Ni(OH)_2 \longrightarrow NiOOH + H^+ + e^- \qquad (5)$$

Immersion of a nickel electrode, or electrodeposited $Ni(OH)_2$ electrode, into an alkaline electrolyte leads to the formation of two crystallographic species, the hydrated α- $Ni(OH)_2$ and anhydrous β-$Ni(OH)_2$ [98,102,103]. The β species is the more stable of the two with a structure of nickel octahedrally coordinated to eight oxygen atoms. The hydrated α structure however is much less crystalline, and has water molecules intercalated between the layers of NiO_2 that form the $Ni(OH)_2$ crystal structure.. On electrooxidation of the nickel hydroxides, two corresponding oxyhydroxide species are formed, γ-NiOOH and β- NiOOH. The former γ structure is relatively unstable, and when oxidised to the oxyhydroxide in an alkaline medium, ionic species intercalate the layers along with water. It can be considered to have the general formula of $A_xH_y(H_2O)_zNiO_2$ (x,y < 1), in which A represents an alkali ion [99,103]. On deposition of the $Ni(OH)_2$ the α and β species are present in non stoichiometric amounts. Potential cycling of the nickel in strong alkali leads to ageing of the material however, characterised by a potential shift to more anodic values [103--105] and an enrichment of the $Ni(OH)_2$ layer.

As with platinum and gold previously, the rate determining step of glucose oxidation at nickel oxy hydroxide is the abstraction of the hydrogen atom at the C_1 atom. This is supported by the rate constant order for amine oxidation at nickel anodes, in which the rate constant decreased in the order of primary>secondary>tertiary [98]. A rate determining step that involved electron transfer would give rise to the reverse order, as an electron is more easily lost from a tertiary amine. Oxidation of all small organic molecules at the nickel electrode occurs immediately after the formation of the Ni(III) species and forms a radical intermediate [98], which in turn reacts with active hydroxyl radicals in the NiOOH surface, as per Scheme 5.



Scheme 5. A suggested mechanism for the oxidation of glucose at a $Ni(OH)_2$ electrode in alkali.
a) $Ni(OH)_2$ is oxidised to catalytically active NiOOH
b) Glucose undergoes hydrogen abstraction at the surface to form a radical intermediate and reforming the $Ni(OH)_2$ species
c) Hydroxyl anions in the solution rapidly complete the oxidation of the organic radical intermediate to form gluconolactone

The reaction product of electrooxidation with the Ni(III) catalyst is still believed to form a gluconolactone and subsequently gluconic acid (Scheme 3). The adsorption of the intermediate is unconfirmed however, as bulk oxidation of glucose seems to occur rather than surface constrained oxidation, as shown by the linear proportionality of peak current to square root of scan rate [3]. Furthermore, repeated CVs of glucose on nickel electrodes show no fouling due to adsorbed intermediates or other species[105--107].

It has been proposed that electrooxidation of organic molecules at NiOOH involves the reaction of 'trapped' hydroxyl radicals in the nickel surface [98]. These are due to the mixed valent and hydrated higher oxide products of equation 5 which are described above to contain alkali ions and water molecules [99,103]. The consumption of the catalytic NiOOH species is evident from cyclic voltammetry of glucose additions as shown in Figure 5 [from [106]]. The $Ni(OH)_2$ modified boron doped diamond electrode shows two distinct redox peaks corresponding to equation 5 in the 1 M KOH medium. However, on 1 mM glucose additions the anodic peak is observed to significantly increase, yet the cathodic peak decreases. This is due to the catalytic regeneration of the $Ni(OH)_2$ species by oxidising the glucose, and as such a relatively smaller current is generated by electrochemical

reduction. Furthermore, the continued catalytic oxidation of the glucose is observed in the negative scan also, until the surface reduction potential is reached and the surface becomes electrocatalytically inactive again. This has been observed in almost all cases of $Ni(OH)_2$ electrocatalysis.

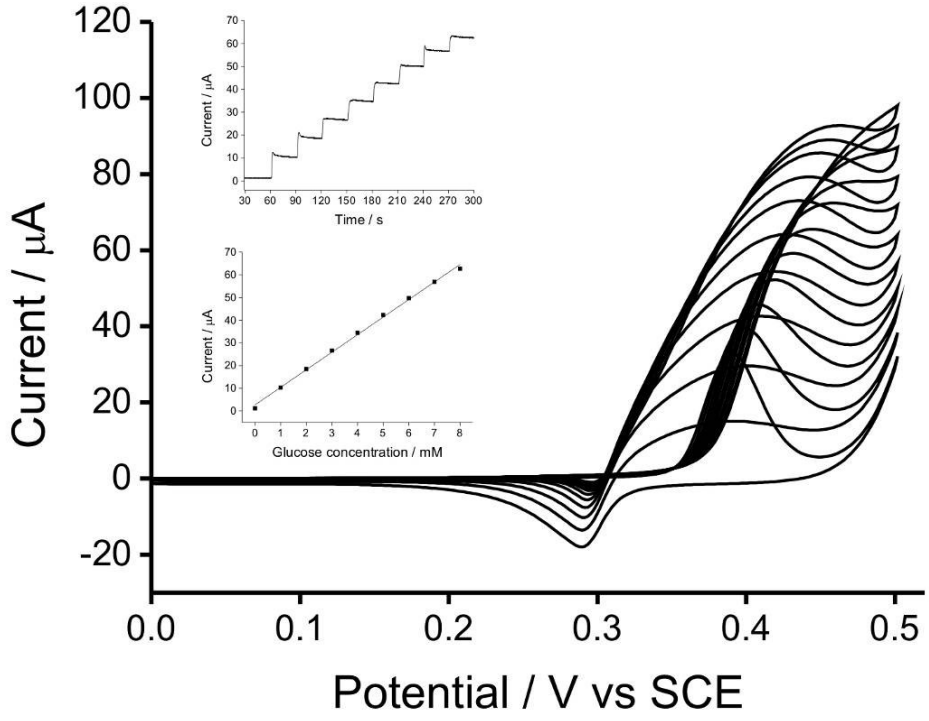


Figure 5. Overlay of CVs following 1 mM additions of glucose to a 1M KOH solution at a Ni microparticle modified boron-doped diamond electrode. In-set: a) amperometric plot of 1 mM glucose additions under constant stirring in 1 M KOH held at 0.48 V and b) the corresponding calibration plot of current vs. glucose concentration. Used with permission from reference [106].

The apparent bulk oxidation of glucose at nickel modified electrodes is possibly due to the bulk presence of the catalytic surface species. Unlike at gold and platinum, the surface is not gradually oxidised with a hydrous premonolayer which eventually forms the oxide monolayer, but instead is immediately oxidised to an oxyhydroxide state at a specific potential.

The loss of electrons at the surface to produce the Ni(III) species seems to instantly activate the nickel electrode indicative of strong oxidant, with suitable empty d-orbitals to rapidly adsorb the organic analyte.

The very high oxidation current produced does suggest that perhaps a more than two electron oxidation of glucose is occurring, yet the shift in the peak with each addition is more indicative of a rapid and prolonged oxidation cycle, and possibly a gradual change in local pH of the electrode surface during the anodic sweep.

3.4.2. Disadvantages of nickel electrodes

Nickel electrodes seem to be the most sensitive of the non-enzymatic electrode materials (see Table 1) with sensitivity reported as high as mA mM^{-1} cm^{-2} in a number of publications [3,106,108]. However they have a number of drawbacks that currently prevent their application to biosensing. Most significant is their inability to function in the physiological condition of approximately pH 7. NiOOH catalysis is highly dependent on the concentrated presence of OH^- anions, as demonstrated by the positive shift of the redox couple and significant decrease in peak currents as pH is decreased [105]. Nickel electrodes have not been reported in this capacity in either pH neutral or acid conditions, and as such their use in glucose sensing is very limited. This is not the case for fuel cells and the food industry however, in which the electrolyte medium is of no consequence to the result, and the higher the catalytic current the better.

Although nickel has proved to be unaffected by chloride ions [107], does not undergo electrode fouling if on a suitable electrode substrate [106,107], and has also shown a promising long term stability, selectivity remains a huge problem. In Fleishmann's [98] investigation of nickel electrodes he found that all the small organic molecules oxidised at the same potential, immediately after the formation of NiOOH. Although most of the analytes explored in that research would not be present in blood (see Table 4), one major oxidisable component often present is ethanol.

Table 4. Possible electroactive and fouling interferences in blood and their relative concentrations

Compound	Blood conc.(mM)[a]	Compound	Blood conc.(mM)
Natural interferents		**External influences**	
Uric acid	0.18 - 0.42	Acetaminophen	0 - 0.2
Ascorbic acid	0.023 - 0.085	Salicylate	0 - 2.2
Bilirubin	up to 0.02	Alcohol (ethanol)	0 - 65
L-Cystine	0.003 - 0.015	L-Dopa	varies
Chloride	98-106	Tolazamide	varies
Protein	6 - 8.4 gm/dL		

Values obtained from Merck manual of medical information [179] a – unless state otherwise

Nickel electrodes oxidise ethanol comparably to glucose as demonstrated in a number of papers [98,104,105,109--111], thus the presence of even low alcohol levels in the blood could affect blood glucose determination. Furthermore, the oxidation potential would be indiscriminate against electroactive interferences such as AA, UA and AP at the fixed potential of amperometric detection, and thus the electrochemical determination would be compromised. This does not also consider a number of other sugars that could potentially be present in the blood depending on the patient's medication.

3.4.3. Recent advances in nickel electrodes

Although not dependent on adsorption to the electrode surface, the surface coverage of NiOOH is essential for efficient and rapid glucose oxidation, and as such a high surface area is preferable. As has been the case for platinum and gold above, the introduction of nanomaterials has shown a significant increase in reported sensitivity and resistance to interference species. Nickel electrodes report the highest current densities for glucose oxidation as shown in Tables 1-3. Hydrothermally fabricated nickel nanoflakes on a titanium support are reported to have a current density of 7.32 mA mM^{-1} cm^{-2}, the highest value reported [3]. In the same region of sensitivity, we see 5.07 mA mM^{-1} cm^{-2} at a nickel film modified nanoporous gold electrode [108], apparently giving a signal 340 times greater than an equivalent nickel film modified polished gold electrode. The simple electrodeposition of nickel/nickel hydroxide onto a boron doped diamond substrate also proved an effective electrocatalytic electrode [106], observing a high sensitivity of 1.04 mA mM^{-1} cm^{-2}, and offering a very simple and reproducible fabrication method.

Ionic liquid pastes have recently incorporated nickel nanomaterials to combine the high rates of electron transfer of the carbon ionic liquid electrode (CILE) with the electrocatalytic effect of nickel hydroxide [112]. The composite electrode offered extraordinary stability, and was easily renewed by light polishing on smooth paper. Different voltammetric behaviour was observed however, in which both the anodic and cathodic peak currents increased with glucose addition, instead of the expected decrease of the cathodic peak. This was also observed at a metallic nickel electrode by Zhao et al [113], and ascribed to the Ni(II)/(III) redox couple serving a double function of electronic medium and catalyst, simultaneously, though what this quite means is unclear. It was not a behaviour observed on another ionic liquid paste electrode described by Zheng et al [60] either, which utilised a multiwall carbon nanotubes (MWCNTs) ionic liquid paste electrode (IL-PE) modified by a nickel (II) quercetin complex.

3.5. Copper electrodes

3.5.1. Mechanism of glucose oxidation on copper

Copper electrodes are often considered in the same way as nickel electrodes with respect to organic molecule electrooxidation. They too catalyse oxidation by reducing the M(III) centre to M(II) (where M = Ni or Cu), however a distinct redox couple for the Cu(II)/(III) surface process is not clearly evident as it is for most nickel electrodes. Fleischmann et al [114] claimed that the copper II/III transition occurs at potentials of 0.65 V vs. SCE in 0.1 M KOH, too close to bulk oxygen evolution to be studied in detail, but there was clear indication of the presence of Cu(III) in the apparent charge under the peaks. The other redox peaks for copper are clearly defined however, with the oxidation to Cu(I) and Cu(II) states, and the corresponding reduction evident in voltammetric studies [115--120].

Kano et al [115,116] investigated the catalytic mechanism for glucose oxidation, and found that a bare copper electrode in 0.15M NaOH was insensitive to carbohydrates. However, a copper (II) oxide electrode responded to the glucose presence with an irreversible oxidation peak. This peak

observed a steady-state type of behaviour at scan rates of 0.1 Vs^{-1} or slower, yet at higher scan rates a definitive peak emerged, suggesting a decreased contribution by a chemical process [115]. The same investigation observed the response of a Cu(I) oxide and $Cu(OH)_2$, and found both to be inactive towards glucose oxidation, thus concluding CuO is necessary to observe catalysis. CuO suspended in a solution of NaOH did not catalyse electrooxidation however, thus suggesting the catalytic component is likely to be a higher oxide such as CuOOH, as is observed for nickel.

Based on comparative RDE experiments of glucose, sorbitol and xylose, Kano et al proposed a 12 electron oxidation of glucose in which the copper oxide facilitates six, two-electron step oxidations to produce formic acid [116]. The complete oxidation of glucose at the copper electrode, and similarly at the nickel electrode, would certainly explain the significantly greater electrooxidation currents observed relative to platinum and gold. Xie et al [117] proposed a mechanism akin to those previously suggested, in which active hydroxyl radicals and the carbohydrate adsorb onto the electrode surface simultaneously, and at adjacent active sites. This is followed by the rate determining concerted step in which the adjacent OH_{ads} abstracts the α-hydrogen atom, and the organic radical is further oxidised by OH^- species to the acid product. Despite this research, the electrooxidation mechanism for glucose on copper remains controversial.

In an experiment by Watanabe et al [121] comparing Cu-BDD and copper electrodes, peak shaped voltammograms were only observed in response to the presence of glucose. Furthermore, the cathodic current of the CV for the Cu-BDD electrode overlayed the anodic current, yet this was not so on the bulk copper electrode. This was believed due to a difference in diffusion domains in which the implanted copper centres of the Cu-BDD electrode imposed a microarray diffusion pattern, whereas the copper electrode had planar diffusion. As such the copper electrode showed a linear relationship with the square root of scan rate, confirming diffusional control of the response, but the Cu-BDD electrode did not.

3.6. Alloys and bimetallic systems

Non-enzymatic electrocatalytic electrodes employing bimetallic systems have been emerging in the form of alloys [120,122--124] or metal adatoms [96,97,125,126], to offer electrodes of highly desirable electronic and catalytic properties. Vasil'ev et al [48] commented on the improved electrooxidation of organic compounds at alloyed platinum electrodes, stating they observed the highest catalytic activity toward small organic molecules. Metal adatoms on electrode substrates, and alloy/bimetallic systems have therefore been the focus of much electrocatalytic research.

The attraction of bimetallic systems stems from the anticipation of a synergistic electronic response, in which the catalytic benefits of each component combine to form a 'super' catalyst [120,122]. Pletcher [44] observed that partial coverage of electrodes, such as platinum and gold, by other metal atoms could greatly enhance catalysis and improve long term activity of the system. An example of the selective poisoning effect of lead adatoms of a platinum surface demonstrates the catalytic enhancement of adatoms, in which the oxidation of formic acid is improved, and long term activity sustained [125,126]. This was proposed to be due to the partial coverage on the electrode such

that the lead atoms interact with two platinum sites, thus forming a surface of pairs of platinum atoms. This is due to the imposed limited active sites across the electrode surface, such that unwanted, poisoning side reactions can not also take place [44].

A variety of Pt-based bimetallic materials (Au, Pb, Bi, Pb and Tl) have been fabricated with the goal of improving the sensitivity and selectivity of the electrodes towards the electro–oxidation of glucose [77,126--128]. The success of bimetallic systems such as PtPb [77,78] and PtIr [76] porous electrode in recent years have already been discussed earlier, again having the advantage of reducing the overvoltage for glucose oxidation to negative potentials. Aoun et al [129,130] explored the benefits of Ag adatoms on single-crystal gold, and observed a marked decrease in oxidation potential that may improve the selectivity and resistance to interferences species across the electrode.

3.7. Carbon based electrodes

Numerous new carbon based electrodes or carbon based modifications to electrodes have emerged over the past decade for use in electrocatalysis, including glucose oxidation. Carbon electrodes have been explored as potential electrodes for direct glucose oxidation for a long time however, with the fabrication of carbon based, screen printed enzymatic electrodes revolutionising finger-stick electrodes in the early 1990s, allowing for mass production and lower cost sensor fabrication [33,34]. Generally carbon substrates are used due to their electronically conductive, yet electrocatalytically inert properties. Glassy carbon electrodes were investigated by Vasil'ev et al [48] in 1985, and found to produce a slight anodic current in response to glucose. This was completely inhibited by the introduction of physiological levels of chloride ions however, thus rendering the electrode nonviable in terms of sensing.

Over the past decade the emergence of carbon-based nanostructured materials such as carbon nanotubes (CNTs), doped diamond-like materials, and most recently graphene electrode materials has opened new doors to improving electrochemical glucose sensing. These materials have great promise in biosensing applications due to their ease of modification and functionalisation, good conductivity, biocompatibility and in some cases, very high surface area. This is particularly so of carbon nanotubes (CNTs), finding application in both enzymatic and non-enzymatic sensors [131--133]. These carbon based electrodes are frequently used as inert substrates, but recent publications also claim that they are directly electrocatalytically active. The following subsections explore boron doped diamond electrodes (BDD), single and multiwall carbon nanotubes (SWCNT and MWCNT) electrodes and other novel electrode materials, such as graphene, as non-enzymatic electrocatalytic glucose sensors.

3.7.1. Boron doped diamond (BDD)

BDD electrodes have found increasing application to electroanalysis over the past 25 years [134--142], including in the oxidation of organic materials. As a substrate electrode for modification, BDD electrodes have been found to be particularly ideal, owing to a very low capacitative current

relative to metal and other carbon electrodes, a highly inert surface and therefore resistance to fouling, and with respect to glucose, a pronounced lack of electrochemical activity [106,143].

A high resistance to electrode fouling is key to the application of BDD towards waste water treatment, as the electrode is durable and resistive to a number of common fouling ionic species such as chloride. It and also has a very large potential window, both cathodically and anodically, due to high oxygen and hydrogen over potentials. Metal modification, by chemical modification [143], metal ion implantation [121,144,145] or electrochemical modification [105,106,146--149], has allowed BDD electrodes to respond to numerous organic analytes effectively and sensitively whilst maintaining low poisoning effects, resilience to harsh experimental conditions and very low background currents.

Despite BDD electrodes being mostly noted far a lack of response to glucose [106,121,144,145,150] the direct oxidation of glucose at an unmodified BDD electrode has also been reported [151--153]. The first report was in 2005 by Lee and Park [152] in which a commercially bought polycrystalline BDD electrode was hydrogen flame annealed prior to experimentation, and used without further modification to oxidise glucose in an alkaline solution. The CV gave an oxidation peak at 650 mV vs. Ag/AgCl for both the forward and reverse scan, in a manner akin to the oxidation of polyamines [154]. In the latter work regarding polyamines it was postulated that the reaction mechanism involved a reaction between adsorbed hydroxyl radicals and adsorbed polyamines, occurring at surface adsorption sites across the BDD electrode. The adsorbed hydroxyl and organic species undergo an anodic oxygen transfer, and the organic compound is oxidised to an intermediate. This is shown in equation 6 in which the electrode adsorption sites are represented by M( ), M' represents a possibly different adsorption site, and R represents the organic analyte.

$$M(OH^{*}) + M'(R) \longrightarrow M( ) + M'(RO) + H^{+} + e^{-} \qquad (6)$$

The organic intermediate was speculated to remain adsorbed on the BDD electrode surface, due to the decrease in the reverse sweep oxidation, and the gradual decrease in peak current over 40 consecutive scans. However, the electrode fouling is to a very small extent relative to other metal electrodes such as platinum and gold, and the current response is readily renewed by simply rinsing the electrode in water.

In Zhao's work [151] the as-prepared microcrystalline and nanocrystalline BDD electrodes reportedly exhibit a high sensitivity and stability, good reproducibility and selectivity over a glucose range of 0.25 to 10 mM in sodium hydroxide solution. The response is surface bound, showing a non-linear response to the square root of scan rate. It is observed to have a rapid increase in peak current at slower scan rates, but at sweep rates over $50 mVs^{-1}$ the peak current is limited and plateaus. This is suggested to be due to slow desorption kinetics of the products at the electrode surface, in which the electrooxidation products do not desorb fast enough to relinquish active sites, thus desorption becomes the peak current limiting factor [151,154]. At sufficient scan rates however, the rate of desorption is exceeded and a maximum peak current is observed.

The BDD electrode response was found to vary from one electrode to the next, indicating an intrinsic difference in the BDD morphology that gave a varied response to the same glucose

concentration. Zhao et al [151] suggest the electrode active sites are in fact the boron atoms heterogeneously dispersed throughout the thin film. They propose adsorption to these electroactive sites, and the subsequent oxidation on the electrode by adsorbed hydroxyl radicals at other surface sites, a theory that indeed supports the varied electroactivity across the different batches of electrodes, and has also been suggested by Koppang et al with regard to the BDD oxidation of polyamines [154].

The effect of electroactive species interferences species UA and AA, were explored by Lee [152] and Zhao [151] on their respective bare BDD electrodes. At AA and UA concentrations as high as 0.5 mM, the oxidation peaks were found to be distinguishable from the glucose peak, and pose no real interference to glucose determination. The electrodes were also utilised in the analysis of glucose in a real blood sample in alkaline solution [155], and a comparable results was obtained.

In both cases, the linear range of the electrode was limited to values up to 10 mM however, a major limitation in the practical use of bare BDD electrodes. The key aspect of the BDD electrode reported in Lee and Park's research is the use of hydrogen flame treatment to maintain the hydrogen termination of the diamond surface. At highly anodic potentials carboxyl functionalities are known to form and decrease the conductivity of the BDD electrode [156]. Consequently the glucose signal is lost at the oxidised BDD surface.

It does remain that BDD electrodes have otherwise shown inactivity towards glucose without first undergoing metal-modification [106,121,143--145,150]. This is possibly due to the surface termination of the electrode, as implied by the inactivity of the oxidised BDD electrode towards glucose [152]. The as-prepared microcrystalline BDD electrode described by Zhao [151] also lacked a response to glucose following acid treatment, yet the nanocrystalline BDD electrode maintained a good response despite 30 minutes of treatment in hot aqua regia.

Thus there is also a strong indication that the surface structure with regard to diamond size, grain boundaries and distribution of boron atoms, has a large effect on the electrochemical activity of the doped diamond electrode. This theory is greatly supported by the recent work of Luo et al [153] in which a boron-doped diamond nanoforest (BDDNF) was fabricated and applied to glucose determination. Relative to a planar BDD electrode, the BDDNF gave a well defined current response to the glucose, a vast improvement that suggests a strong dependency on the surface structure of the electrode. All the unmodified BDD electrodes used in direct glucose oxidation discussed here have shown selective determination of the analyte in the presence of ascorbic acid and uric acid. This is in part due to a significant difference in peak potential, in which in all three publications [151--153] the interferences respond at approximately 100 to 300 mV vs. SCE, where as the glucose has a peak current at approximately 600 mV vs. SCE.

However, in each case the linear range of each electrode does not exceed 10 mM, which is a major issue with regard to developing a viable biological glucose sensor. The sensitivity of the electrodes is also lower than at metal modified electrodes, including metal modified BDD electrodes [106], observing values of 101.9 $\mu A\ mM^{-1}\ cm^{-2}$ on the BDND [151] and merely 8.1 $\mu A\ mM^{-1}\ cm^{-2}$ on the BDDNF electrode [153].

These are very recent publications however, and it is therefore evident that nanocrystalline and nanostructured boron doped diamond materials hold a lot of promise as viable, reusable glucose sensors for diabetes.

3.7.2. Carbon nanotubes

Carbon nanotubes (CNTs) are also a relatively new carbon based electrode material that offers alternative electroactivity to conventional carbon surfaces. CNTs have found substantial application to electroanalysis of a wide variety of analytes, most frequently in the modification of planar electrodes, increasing surface roughness, porosity and compatibility to biological substances. The cylindrical graphene sheets of nano dimensions are highly conductive, and substantially increase the surface area of a planar electrode whilst retaining high chemical and physical stability. In Table 1 multiwalled and single walled carbon nanotubes (MWCNTs and SWCNTs) are shown to have been used alone [157,158] but most frequently in conjunction with other catalytically active metals [54,56,59,60,128,159--163].

As mentioned in section 2.4, carbon nanotubes have shown promise in glucose sensing in the development of third generation enzymatic glucose sensors [164,165]. When immobilised with GOx, the carbon material does not require a mediator or oxygen to facilitate glucose oxidation, and as such is believed to allow for direct electron transfer between the enzyme and electrode [164]. Furthermore, the CNT modified electrodes have shown greater long term stability than is observed on a modified glassy carbon electrode [165], due to the strong adherence of the enzyme to the carboxylated open ends of the nanotubes [164].

Non-enzymatic and unmodified carbon nanotubes have been also been successfully used in glucose detection, as reported by Ye et al [157]. A well-aligned MWCNTs electrode was fabricated on a tantalum substrate grown on a thin layer of cobalt catalyst. In alkaline conditions, and clear faradaic response to the presence of glucose was observed at 0.42 V vs. Ag/AgCl, and a diffusion-controlled process was indicated by the linearity of the square-root of scan rate vs. peak current plot [157]. The cobalt present in the fabrication process was found to contribute approx. 14% of the glucose response however, but the bulk of the response was considered to be due to the CNTs. Although the CNTs gave a good, linear response to glucose to levels of 11 mM, even in high concentrations of chloride, the chronoamperometric response to interference species, AA and UA, was considerable, accounting for 25 % of the current response relative to glucose. This is possibly due to the lower overpotential of 0.4 V vs. Ag/AgCl used with the CNTs compared to the ca. 0.6 V potential applied to the BDD electrodes, in which the interfering species oxidized at lower potentials to glucose. Wang et al [158] fabricated free standing SWCNT films in a more recent investigation, in which carbon nanotubes were spread of a glass substrate and bound to the surface using Nafion. In this instance the CV showed an increase in current from approximately 0.5 V vs. Ag/AgCl, though no discernable peak was produced. The linear range was also limited, deviating at the very low concentration of 2.16 mM.

The nanotubes subsection of Table 1 includes numerous CNTs-based electrodes that have been modified with various catalytic metals, most of which have been discussed previously. Sensitivity at best is reportedly 280 $\mu A\ mM^{-1}\ cm^2$ for a platinum nanocube doped CNTs electrode [55], and linear ranges rarely exceed 15 mM. Platinum and platinum group bimetallic nanoparticles have been successfully distributed on CNTs in a number of publications, and have offered a catalytic response without the issues of electrode fouling [54--57,159,166].

A number of metal oxides have been used in conjunction with carbon nanotubes to produce electrocatalytic glucose sensors [55,58,160,167--169]. MnO_2 was coupled with carbon nanotubes in 2008 by Chen et al [160] in a novel nonenzymatic glucose sensor, which showed a wide linear range to 28 mM and a reasonable sensitivity of 33.19 $\mu A\ mM^{-1}\ cm^{-2}$. The alternative metal oxide displayed a high tolerance to electrode fouling too, exhibiting no interference from chloride ions or common electroactive interference species. Most notably the MnO_2 caused a substantial negative shift of the anodic peak potential to +0.1 and +0.4 V vs. Ag/AgCl, a shift of over 200 mV relative to the unmodified MWCNTs. Two oxidation peaks were observed in the anodic sweep of the MnO_2/MWCNTs electrode before glucose addition, the first assigned to the Mn(II) to MnO_2 oxidation, and the second to the MnO_2 to MnO_4^{2-} process. Both peaks significantly increased on addition of glucose, and as such it was suggested that the both Mn(II) and Mn(IV) were involved in the electrocatalytic process [160]. CuS_2 and SnO_2 were deposited on double wall carbon nanotubes, though the latter gave a relatively poor response to glucose and H_2O_2, and the CuS_2 was only marginally better. The latter experiment did strongly emphasize the catalytic nature of copper towards glucose oxidation however, as the SnO_2 was speculated to be most active due to the presence of more active sites, yet this was evidently not the case.

Overall, carbon nanotubes have been shown to offer a good base for simple modification, to allow for deposition of less material, yet still offer high catalytic response and electron transfer. Furthermore, unmodified CNTs are directly responsive to glucose, and unaffected by interfering species and chloride, as well as resistant to surface fouling. However, a major limitation lies in the linear range of both modified and unmodified CNTs, as the latter do not exceed 10 mM and the former rarely surpass a 15 mM maximum.

3.7.3. Graphene and carbon nanofibres

A graphene nanosheet (GNS) is the basic structural component of graphite, carbon nanotubes, fullerenes and carbon nanofibres. It is a planar 2D layer, a single atom thick, of sp^2 hybridised carbon, and was first fabricated and discussed in 2004 by Novoselov et al [170]. It was long believed that graphene could not exist in its free-state, due to thermodynamic instability, instead preferring to form more stable, curved structures. However, on successful fabrication by exfoliating small flat elevated areas of highly oriented pyrolytic graphite (HOPG), the unique electrochemical properties of the material were discovered [170]. GNS is unique as theoretically it is a zero bandgap semiconductor [170] therefore has very high conductivity akin to that of metal, a property unknown to films of such thickness. The new carbon structure is therefore considered to possibly offer a new wave of electrocatalytic abilities, particularly in combination with metallic nanoparticles [171]. That said, work by Pumera [172] rightly suggests this excitement might be tempered with caution. Recent advancements in the preparation of graphene have allowed experimental studies of graphene to rapidly progress, and as such the last two years have seen a surge in graphene based catalysts. With regard to glucose oxidation, experiments thus far have focused on enzymatic systems, in which GOx is immobilised on the surface [173--177], along with nanoparticles of platinum and gold. As yet there have not been any non-enzymatic studies regarding graphene based electrodes however. That said, the

attempted step from enzymatic to non-enzymatic approaches can be anticipated. It has been speculated that sp^2 carbon plays an important role in the electrochemical detection of organic compounds, due to the low potential at which the hydroxyl radical may be formed at the surface sites [154]. As such there is further indication that non-enzymatic glucose sensing might be possible at graphene thin layers.

In a variation on graphene, carbon nanofibres (CNFs) are finding increasing application to biosensors, including enzymatic glucose sensors. Carbon nanofibres are composed of well-arranged graphite layers that are organised into cylindrical structures to give highly conductive nanowires. Unlike carbon nanotubes, the fibres are not hollow, and expose the edges of the graphene plane rather than the basal plane surface, thus they have a very large active surface area and a very conductive [178]. In the past 4 years, carbon nanofibres have been modified with enzymes to produce successful glucose biosensors with superior performance to modified carbon nanotubes. Non-enzymatic CNF electrodes have also been developed recently however. Rathod et al [159] modified the CNFs with a fine distribution of platinum nanoparticles, and analysed glucose under physiological conditions (pH 7), though the platinum modified MWCNTs proved to be a better sensor. This surprising result was attributed to a relatively lower level of platinum doping and accessibility of particles for glucose oxidation due to the larger size of the CNFs used, as well as the effect of coating the surface with an insulating Nafion layer [159]. Liu et al [107] developed a renewable nickel nanoparticle loaded carbon nanofiber paste electrode, in which a wide linear range of 2μM to 2.5 mM was observed, and a sensitivity of 420.4 $\mu A\ mM^{-1}\ cm^{-2}$.

4. ELECTROACTIVE INTERFERENCES AND ELECTRODE FOULING

Two major problems of the amperometric glucose sensing is the presence of other electroactive interferences in the blood and electrode fouling. The moderately high anodic potential used to oxidise glucose also oxidises these interferences, thus giving an amperometric current that overestimates the blood glucose value. This also applies to non-enzymatic glucose sensors, in which selectivity is even less, and electrooxidation reactions are enhanced. The overestimation of blood glucose levels might have life threatening consequences, as hypoglycaemia can be masked or even be induced by inappropriate administration of insulin, thus leading to a hypoglycaemic coma or even death. A number of compounds found in human blood can interfere with the glucose determination. These are listed in Tables 4 and 5 and discussed in the following sections.

Table 5. Saccharides other than glucose that have known and possible interfering electrochemical responses to electrochemical glucose sensors

Sugars	**Source**	
Know interferences		**Possible interferences**
Maltose	food, immune globulins, perineal dialysis	Mannose
Galactose	food	Lactose
Xylose	malabsorption testing	Ribose
Icodextrin	perineal dialysis	Arabinose

4.1. Naturally occurring interferences

Ascorbic acid (AA) and uric acid (UA) (Figures 6a and b) are two electroactive species naturally occurring in blood that cause the most electrochemical interference during glucose sensing.

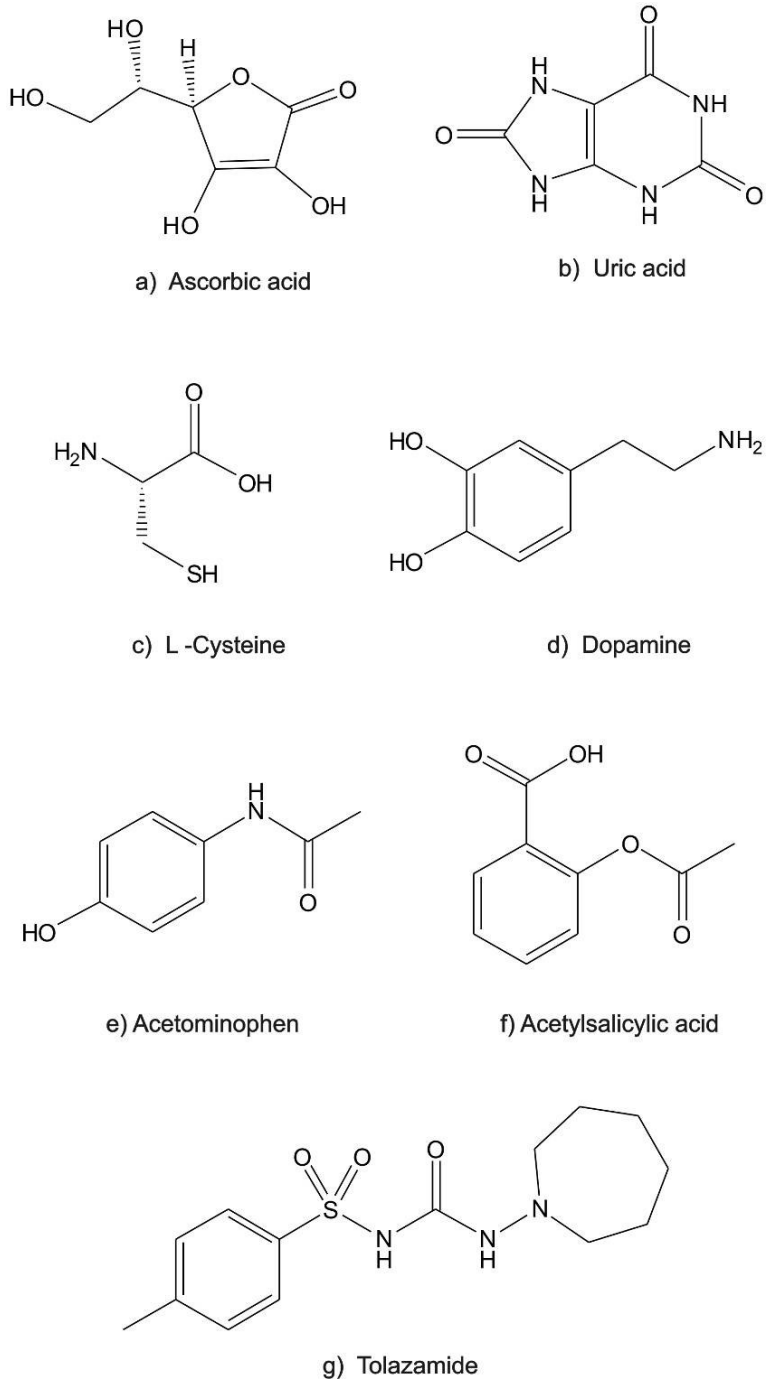


Figure 6. The chemical structures of various known electroactive interferences species. a) Ascorbic acid, b) uric acid, c) L-cysteine, d) dopamine, e) acetaminophen, f)acetylsalicylic acid and g) Tolazamide

Like glucose they are strong reducing agents, therefore readily oxidise at moderate electrode potentials. AA is commonly known as vitamin C, and although concentrations in blood vary on dietary intake, normal blood concentrations are ca. 0.02 and 0.08 mM [179]. UA is present as the final metabolite of purine, and is present in the blood as an antioxidant at concentrations of ca. 0.18 to 0.42 mM. Ever present, most recent papers of the past decade focus on these two main electroactive interference compounds when testing the practical application of the sensor, be it enzymatic or non-enzymatic.

Cysteine (Figure 6c) has been named as an electroactive interference with regard to glucose sensing by a number of researchers [180--184], and is present in the blood at very low concentrations of 3 to 15 μM. Dopamine (Figure 6d) is also present at low levels naturally, and is known to oxidise at similar potentials to glucose [113,185,186] thus often considered a natural interference.

As can be seen in Table 4, the concentrations of the interferences are found in sub millimolar concentrations, which is considerably lower (ca. 250 times) than the blood glucose concentration of 3-8 mM (optimally). This can arise if the interferences react in a diffusion controlled manner where as the glucose is subject to kinetic limitations at the potential of interest.

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4.2. Pharmacological interferences

A number of drugs, ranging from commonly used to highly specific, have been shown to interfere with blood glucose determination. The most damaging of these is acetaminophen (AP) (Figure 6e), a constituent of paracetamol and therefore commonly used and of a variable concentration in the blood, with therapeutic levels of up to 0.2 mM. Similarly, salicylic acid (Figure 6f), a component of aspirin, may also be present in the blood at an interfering level, though actual interference by salicylates has not been generally reported at enzymatic glucose sensors. Another common interference species is dopamine, naturally occurring in low concentrations, yet found in certain food stuffs and administered as a drug in the form of its precursor L-DOPA.

Finally one other recognised interfering chemical that has had variable effects on GOx based sensors is ironically Tolazamide (Figure 6g), a sulphonamide based drug used to lower blood glucose levels in Type 2 diabetics. These are all chemicals from drugs known to interfere with enzymatic blood glucose sensors, yet potential interference by many drugs that is yet unknown must always be considered, especially as new chemicals and compounds are always being produced in the pharmaceutical industry. The impact of these chemicals on non-enzymatic glucose sensors is much less researched, as non-enzymatic blood glucose monitoring systems have yet to be introduced. As such, the impact of so many drugs needs to be determined, especially as electrooxidation at non-enzymatic systems is most frequently not selective and produces a greater oxidative current response.

4.3. Proteins and chloride anions

Interferences also include proteins, of which there are can be up to 8.4 g/dL in the blood, which readily adsorb and cause inhibitory electrode fouling, particularly on platinum and gold electrodes

[38,48]. High concentrations of triglycerides in the blood can have a similar effect, competing with glucose in the sample volume, thus lowering the glucose oxidation signal. Chloride anions greatly interfere with platinum electrooxidation signals, as the chloride strongly chemisorbs irreversibly onto the platinum surface, and thus inhibits catalysis [38,48]. Chloride concentration is relatively high in the blood, with a concentration of approximately 100 mM, and thus its poisoning influence can not be deemed negligible.

Phosphate buffers, in which much of the pH neutral experimentation is performed, are believed to have a somewhat interfering effect themselves on platinum electrodes [48,67]. Although not an interference present in blood samples, the effect of phosphate anions is not to be disregarded, as many preliminary experiments are performed in the buffer solution to give an idea of the electrode response in a real sample.

4.4. Non-glucose sugars

Although glucose is the main carbohydrate found in blood, a number of other mono and polysaccharides, listed in Table 5, may be present that can severely interfere with glucose analysis [187]. The presence of non-glucose sugars in the blood has proved problematic for enzymatic sensors in spite of their higher selectivity towards glucose relative to non-enzymatic systems [187]. In 2003 a medical device alert was issued for glucose monitoring systems that used glucose dehydrogenase (GDH)-pyrroloquinolinequinone (PQQ) enzymatic test strips. The interference sugars were identified to be icodextrin and maltose, the former a polymer of glucose consisting of 10 to 250 glucose units, and the latter, a metabolite of icodextrin consisting of two glucose units. Icodextrin is used in an ever increasing demand for peritoneal dialysis in the treatment of chronic kidney disease. On the death of a number of patients investigation into the effects of non-glucose sugars on blood glucose determination found monosaccharides galactose and xylose, to interfere with blood glucose monitoring systems that were based on glucose dye oxidoreductase and GDH-PQQ enzymatic test strips. GOx based test strips have also shown interference by galactose and maltose [187] resulting in an overestimation of blood glucose levels, though they are generally considered to not be susceptible to non-glucose sugar interference. Only systems based on GDH-nicotinamide adenine dinucleotide (GDH-NAD), or GDH-flavin adenine dinucleotide (GDH-FAD) are free of these interferences.

4.5. Alcohol

One major concern for the application of non-enzymatic glucose sensors is the potential interference of alcohol. A similar catalytic response by many metallic, non-enzymatic electrodes to alcohol is a major issue, as many of the electrodes presented as possible glucose sensors have also been applied to alcohol sensing in similar arrangements [104,188--192]. Direct alcohol fuel cells that frequently employ ethanol, are based around similar electrode systems to non-enzymatic glucose electrodes. In fact, a number of potential glucose sensors are suggested in glucose fuel cells, due to the high oxidative current produced at certain catalytic metals.

4.6. Methods to overcome interference effects

In industry and laboratory based testing, FIA and HPLC systems can be used to separate different sugars and alcohols prior to oxidation at the non-enzymatic electrode, thus avoiding the issue of interferences, as shown in Figure 7.

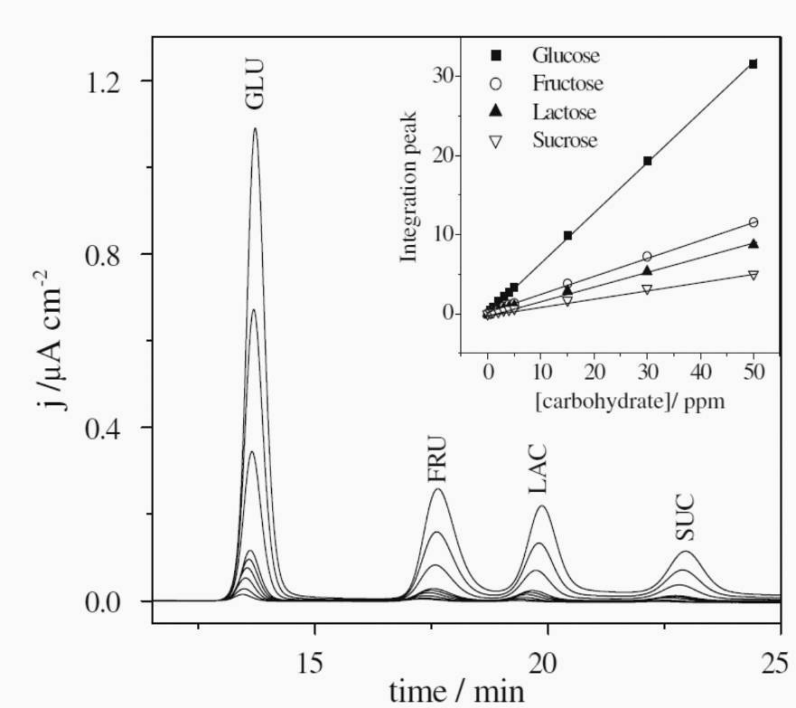

Figure 7. The determination of different sugars using HPLC methods. Amperometric detection was performed using a nickel hydroxide modified electrode. The solutions were passed through chromatographic column. Eluent: NaOH 0.1 M, flow rate: 1.0 mL min^{-1}, electrode potential 0.45 V vs.Ag/AgCl. Reproduced with permission from [225].

This is not possible for blood glucose sensors however, as chromatographic systems are not viable in terms of self testing using in-vitro handheld glucose meters, and they are certainly incompatible for in-vivo analysis.

A number of approaches have been taken to overcome the problem of interferences. These include the use of permselective coatings such as Nafion [57,70,193,194] or electropolymerised films [12,184,195], reducing the overpotential for glucose detection to less anodic values at which the interferences are not oxidised [76--78,96,97], the use of chromatographic stages prior to analysis (Table 2), and the specific fabrication of the electrode surface to enhance the kinetics of the slower, surface bound reaction.

The option to use a passive membrane that imparts chemical selectivity and size exclusion to retard the interfering species is the most well established approach. Permselectivity leads to a

significant improvement of sensitivity, selectivity and stability of the electrochemical sensor. Nafion is a sulfonated tetrafluoroethylene copolymer, and imparts a negative charge that discriminatively prevents the transfer of anions and electrons, but allows the transport of cations. Their application to biosensors has been extensive [57,70,193,194], as they are an effective way of preventing electrode fouling and imposing some selectivity for glucose catalysis [184]. Undesirable anions such as organic acids (AA, UA), chloride anions and phosphate anions can be suitably repelled by the charge exclusive Nafion film, which greatly limits their transport to the electrode surface. Electropolymerised permselective coatings, such as polyphenol and overoxidised polypyrole, impart size exclusion as well as charge repulsion, so that macromolecules such as proteins are unable to foul the electrode surface. Electropolymerization offers several advantages as well, such as the simplicity of the experimental setup, ease of control and capability to address the modification solely on the device sensor [184,196].

Reduction of the oxidation overpotential to values cathodic of the interference oxidation is another option. Generally metal and metal oxide electrodes oxidise glucose at potentials of 0.4 to 0.7 V vs. SCE. This is a relatively high potential, and is therefore capable of oxidising a number of available species. The glucose active overpotential of gold electrodes has been successfully reduced by modification with partial monolayers of adatoms such as silver [77,96]. Oxidation peak potentials of ca. -0.5 V vs. Ag/AgCl were observed on the 1/3 Ag monolayer on Au(100) electrode. This was also observed for a porous PtPb electrode [77] in which the overpotential was reduced to -0.08V vs. Ag/AgCl and successfully avoided interference from AA, UA and AP.

In this latter case, the structure of the electrode may also have played a part in improving electroselectivity towards glucose. As discussed in the previous section, the surge in fabrication of nanomaterials has led to a dramatic increase in viable non-enzymatic glucose sensors. The nano dimensions of a sensor would theoretically aide the sluggish kinetics of the electrooxidation of glucose, by enhancing surface bound reactions and making no difference to mass transport limited electrode processes such as AA oxidation. This has been observed in a number of publications of late, and has demonstrated that a reduction in oxidation potential is not necessarily needed [68,153,197].

5. THE REQUIREMENTS OF GLUCOSE MONITORING

Blood glucose monitoring (BGM) is one of the main applications of glucose sensors. Three commercial and clinical systems are presently in place, all involving enzymatic approaches. One accurate approach is known as the hexakinase method, but it is a lab based approach and not viable for portable, self-testing BGM. The other methods, as discussed in Section 2, involve enzymatic electrochemical glucose sensors based on either glucose oxidase or glucose dehydrogenase as their catalytic component. However a number of factors affect BGM that need addressing and overcoming as the technology evolves.

At present the main method of monitoring blood glucose levels is to self-test a blood sample taken from a finger prick or similar. This has been the established method for a long time, and allows the patient to take a sample reading of their blood glucose levels at various points through out the day. Having an in-vitro system also allows for more liberties with respect to the electrode set-up, in that

biological considerations such as immune response and toxicity are negligible. Another approach to blood glucose monitoring is the use of in-vivo glucose sensors for continuous glucose sensing. This approach would give glucose concentrations in real time, and provide a better overall understanding of blood glucose fluctuations.

5.1. In vitro glucose monitoring: Single point 'fingerstick' glucose sensors

At present fingerstick glucose sensors are disposable, screen printed enzyme electrode strips, and are produced by a variety of companies including Abbott Diabetes Care [198], AccuChek [199], LifeScan [200] and Bayer [201]. The fabrication process for these disposable sensors involves mass production of a complex mixture of enzymes, mediators and stabilisers which is screen printed in a thick film onto a suitable substrate. The electrode system is self-contained with a working and reference electrode, and as such a small blood sample should cover the entire set up and provide its own electrolyte. Blood samples range from 0.3 to 1.5 μL for modern testing strips, considerably less than the 3 to 10 μL samples required merely 10 years ago [202] thus indicating the rapid advancement and sophistication of fabrication methods and biosensor technology.

Table 6. Parameters of commercially available enzymatic glucose sensors

Manufacturer	Model	Range (mM)	Enzyme	Sample size (μL)	Test t (s)	Oper. T	Oper humid	Haematocrit	Mediator
Lifescan	OneTouch UltraEasy	1.1 - 33.3	GOx	1	5	6– 44°C	10-90 %	30 - 55%	--
AccuChek	Aviva	1.0 - 30.0	GDH-PQQ	0.6	5	6 – 44°C	10-90 %	20 - 70%	$Fe(CN)_6^{3-}$
Abbott	FreeStyle Lite	1.1 - 27.8	GDH-FAD	0.3	5	4 - 40°C	5% to 90%	15 - 65%	Os 2+/3+
Bayer	Ascensia Contour	1.0 - 30.0	GDH-FAD	0.6	5	5 - 45°C	10 - 93%	--	$Fe(CN)_6^{3-}$

Table 6 lists the commercially available single use fingerstick strips of four of the companies involved in their production. The table compares the electrochemical assay, glucose range, temperature and humidity dependence, sample size and testing time. As can be seen, only the LifeScan Onetouch employs the glucose oxidase enzyme in its sensor. GDH based enzyme electrodes have gradually replaced GOx systems in the past decade due to the error imparted by variable oxygen levels on the latter approach. GDH enzymes are not influenced by oxygen, yet are less substrate specific and stable. The substrate specify issues of GDH-PQQ were of particular concern at the turn of the 20th century, as discussed in Section 4.3 with regard to possible overestimations of blood glucose in patients receiving treatments such as peritoneal dialysis. In 2006 Tsujimura et al [16] reported a novel FAD dependent glucose dehydrogenase (GDH-FAD) enzyme, which was used in collaboration with a ferricyanide mediator. The resulting sensor gave a linear range of 5-30 mM, a highly selective amperometric response to glucose, was insensitive to oxygen, and also showed negligible response to other saccharides. This is employed by Abbott in their most recent Freestyle strip range [20].

Although they are the only commercially available systems for BGM, enzyme based glucose sensors have numerous intrinsic flaws and numerous sources of errors. A recent review by Ginsberg [203] explores the factors affecting blood glucose monitoring, in which he identifies four main sources; strip factors, physical factors, patient factors and pharmacological factors.

Strip factors include the surface area coverage of enzyme in the sample wells, the use of a mediator which is unstable in the oxidised form and at high temperatures, and the detrimental affect of high temperatures and high humidity on the performance and lifetime of the strips. The thermal instability is problematic to fabrication as well as storage methods, and NAD centred GDH requires a more complicated oxidation system to GOx and PQQ [16]. With regard to physical factors, the effect of altitude and temperature were explored to test the accuracy of various test strips. It was found that altitude affected GOx based meters by 6-15 %, where as the non-oxygen dependent GDH systems observed accuracy within 5%. On testing at a temperature of 8°C the accuracy of the five strips tested varied considerably, regardless of the enzyme base with errors of 5-7% either positively or negatively [203].

Patient error is more difficult to address, as it involves miss use of the glucose monitor, and variation in haematocrit, the ratio of red blood cells to plasma in the whole blood. The former can be resolved by appropriate design and instruction of the meter, yet the latter is the percentage level of red blood cells in the blood, which varies considerably. Serious errors in BGM arise from variable haematocrit levels, with studies showing an error as large as 40% at very low haematocrit [204]. Lab based laboratory tests monitor plasma glucose levels rather than whole blood, thus observing glucose levels generally 10-15% greater than in the whole blood. Self BGM can not be as specific, and tests the whole blood, therefore needs to account for variable haematocrit in its design.

In a bid to reduce patient discomfort in sample acquisition blood sample sizes have vastly decreased and are now of sub microlitre volumes. However, on the reduction of the sample size, the margin for error by contamination is increased. As Ginsberg discusses [203], if the patient has not washed their hands a contamination of just 1 µg of glucose could raise the glucose reading of a 0.3 µL blood sample by 300 mg/dL. This is an error perhaps best addressed by educating patients rather than attempting to account for external surface contamination in the sensor. This is an approach that would suit addressing pharmacological errors also, as were discussed in Section 4 and include the use of prescription drugs and non-glucose sugar treatments.

The introduction of ‘wired’ enzyme electrodes by Heller et al completely altered the conventional approach to glucose sensing [19]. Heller et al presented an innovative design that used parallel facing carbon working electrode and Ag/AgCl counter electrode, merely 50 µm apart [20,22]. This reduced the electrode area, and produced a faster assay, as well as imposing a strict control on the sample volume. To overcome the problem of a large capacitance due to rapid electron shuttling between the facing electrodes, a wired enzymatic system was proposed that utilised an $Os^{2+/3+}$ redox mediator with a formal potential between -0.2 and 0.0 V relative to the Ag/AgCl counter electrode. As such, the oxidised mediator could only be reduced by the enzyme thus preventing a current to be passed within the electrode cell. The very small sample volume also ensures the complete, rapid oxidation of glucose, as opposed to partial oxidation of glucose or mediator as observed in other designs, therefore a more accurate determination of blood glucose is possible.

5.2. In vivo glucose monitoring: Continuous glucose sensors

Single point fingerstick glucose sensors have significantly improved diabetes control and blood glucose management over the past four decades. However, a number of clinical trials have shown that tighter glycemic control is required to obtain a significant reduction in complications related to diabetes [205--207]. Although instantaneously accurate, intermittent in vitro blood testing does not give a suitable picture of glycemic behaviour, providing a static overview of a continually changing system. In particular the time spent in high or low glycemic episodes is grossly misrepresented, and as such a gradual deterioration in health is still observed. The answer to this problem is to increase the frequency of blood glucose sampling, without however, increasing the frequency of the painful and inconvenient fingerstick monitoring. Over the past five years continuous glucose monitors have emerged to achieve exactly this.

Continuous glucose monitors (CGM) are in vivo electrochemical sensors inserted into the subcutaneous tissue of the upper arm or stomach for a period a days. During this time the glucose level of the interstitial fluid is continually monitored with readings taken as frequently as every minute and calibrated daily by a fingerstick strip test. The amperometric signal is recorded and the data is transferred wirelessly to a receiver which then assesses the glycemic levels. The original CGMs were analysed retrospectively by health workers performing 3-day blind studies. In 2000 the first real time continuous glucose monitor was commercialised [208], yet the past decade has seen a significant improvement in CGM technology. Presently, three CGM systems are available on the market; the Freestyle Navigator (Abbot Diabetes Care), Guardian ® Real-Time (MiniMed), and DexCom Seven. In a recent study [209] the numerical and clinical accuracy of the CGM systems were compared, concluding that the Navigator and Guardian had comparable numerical accuracy but the DexCom observed a 30% higher value.

CGM systems pose additional complications to the issues associated with in vitro testing. First, the electrode needs to be able to undergo constant, stable and accurate amperometric detection, without undergoing electrode fouling, deterioration of the surface, or saturation of the surface. Secondly, the electrode must be very small so as to achieve the least discomfort possible. Thirdly, they must be completely non-toxic, without the possibility of any leachable materials. Finally, the sensor must be biocompatible. That is to say, it must cause the minimal immune response, as inflammation of the insertion site and surrounding tissue will produce a misrepresentation of the blood glucose, and significantly increase the risk of biofouling [210].

One of the most successful CGMs currently in use is the Abbot Freestyle Navigator [206,209], and it accounts for the above complications in a number of innovative ways. It consists of a 0.6 mm wide plastic substrate in which the working, reference and counter electrodes are layered by screen printing [20]. On the carbon working electrode the wired enzyme technology is once again utilised, and is covered by a flux-limiting membrane. Unlike the strip technology, the CGM sensor uses a GOx-FAD enzyme centre. This is combined with an osmium redox mediator using a conductive hydrogel which binds the electroactive components to the sensor, yet allows for the diffusion of glucose, glucolactone and water soluble species [5,20]. One of the most important properties of the sensor is the bound redox mediator, which therefore renders the sensor non leachable. The use of

$Os^{2+/3+}$ allows for the application of a low potential of just 0.04 V vs. Ag/AgCl. The CGM has been successfully administered for over 5 days in trials [206], and requires no more than a one-point calibration from a drawn sample once a day.

6. REQUIREMENTS OF NON-ENZYMATIC BLOOD GLUCOSE SENSORS

The intrinsic instability of enzymes and oxygen limitation encountered in glucose monitoring strips are two persistent and significant problems with commercial, in-vitro glucose sensors. Elimination of the bio-component and dependence on oxygen is therefore a desirable advancement of glucose sensors, and is the reason practical and viable non-enzymatic glucose sensors that supersede modern, commercially available systems are so highly sought after.

Table 7. The minimum requirements of a viable glucose sensor

Minimum requirements of a blood glucose sensor	
Property	*Reason and/or required minimum response*
Selectivity	Must be selective to glucose only, with min. interference from erroneous sources
Sensitivity	1μA cm^{-1} mM^{-1} or greater to improve on enzymatic sensors
No oxygen dependency	A reliance on oxygen is a major problem for GOx systems
Stability	Stable during application and have a lifetime exceeding 6 months
Accuracy and precision	Must meet ISO* standards of within 20% error 95% of the time
Biocompatibility	Particularly for in-vivo use due to biological components and immune response
Low cost	Fabrication costs and running costs must be lower than enzymatic systems
Easy fabrication	Easier fabrication than screen printed enzymatic electrodes strips

* ISO – International Organisation for Standardisation

The list provided in Table 7 outlines the minimum requirements (in addition to the data in Table 6) of non-enzymatic glucose sensors if they are to compete or indeed replace the enzymatic industry. The main areas for concern are selectivity, which is evidently still a problem for enzymatic systems, and sensitivity in a specific media i.e. blood at pH ca. 7.4.

At present research regarding chemical interferences at non-enzymatic electrodes is positive, with increasing selectivity being observed with regards to AA and UA in particular [76,112,153,211]. Less research has been conducted into the effects of non-glucose sugars however [96,212,213]. Aoun et al found that a gold electrode single crystal electrode modified with a silver ad-layer gave a response dependent on the sugar structure [96]. Glucose is an aldose type sugar, along with galactose, xylose, ribose, arabinose and mannose to name a few. They are monosaccharides which possess one aldehyde group per molecule, and therefore they all posses a single hemiacetalic carbon atom. Aoun found that only the aldose type monosaccharides, and also the aldose disaccharides maltose and lactose, gave a

significant oxidation current. Ketose type monosaccharides, in which the molecule has a single ketone group, did not oxidise at the modified gold electrode [96]. Choudhry et al [212] found a copper oxide screen printed electrode to have a modest selectivity for glucose over maltose, sucrose and fructose, with the latter not giving any response, and the former two, both containing a glucose molecule, gave oxidative responses at potentials 0.39 and 0.46 V more positive of the glucose response respectively.

Sensitivity is considerably greater at non-enzymatic electrodes, with enzymatic systems stating 'high currents' of approximately 5 $\mu AmM^{-1}cm^{-2}$ [30,177,214,215], relative to currents of generally 100 $\mu AmM^{-1}cm^{-2}$ or more reported at non-enzymatic sensors.

With regard to the ever-decreasing sample size, the potential for nano-dimensioned, non-enzymatic electrodes far exceeds the constraints of large macrosized enzymes, potentially allowing for even smaller sample sizes and higher sensitivity. The benefits of inorganic fabrication methods also compete favourably against biosensor fabrication, as the delicate nature of the enzyme need not be considered, thus allowing for more diverse, rapid and lower cost processes. This offers another advantage to non-enzymatic glucose sensing; the ability to reuse an electrode. The long term stability of non-enzymatic electrodes varies widely depending on the fabrication method. Yet sustained, relatively high sensitivity has been observed and non-enzymatic glucose electrodes and their renewable properties demonstrated [107,112,152,216]. This may have a more significant application to in-vivo glucose monitoring, though renewable and robust in vitro sensors would also be cost effective and practical.

Although not the case for all non-enzymatic electrodes discussed here, a large number require an alkaline medium to operate [96,106,122,163,217--220], and as such non-enzymatic electrodes face their greatest problem in biocompatibility. For the most part, this applies to nickel and copper electrodes, with the rapid development of carbon based non-enzymatic electrodes is moving towards more biologically compatible sensors.

7. OTHER APPLICATIONS OF NON-ENZYMATIC GLUCOSE ELECTRODES

Glucose electro-oxidation is not only useful to blood glucose monitoring but may be applied to other areas such as the food industry, industrial processes, and the development of fuel cells and batteries. Although they have a potential for use in the blood glucose industry, non-enzymatic sensors are possibly of greater use in non-physiological situations. This is primarily due to their lower selectivity, as industrial systems have a greater opportunity to impose selectivity via chromatographic methods, or indeed they may involve 'clean' systems which do not contain interference species. The high electrocatalytic activity of non-enzymatic electrodes vastly outweighs that of their enzymatic counterparts, and as such they are potentially useful in the fuel cell industry. Furthermore, the intrinsic thermal stability, chemical resistance and long term sensitivity observed in a number of non-enzymatic systems place them in a better position for experimentally extreme environments.

7.1. Industrial processes

In their application as sensors, non-enzymatic glucose electrodes may find extensive application as sensors in bioindustrial processes. Such industry includes fermentation plants, in which strict environmental controls are in place for optimum production. The need for diverse and adaptable sensors with a good, stable performance in variable conditions is therefore required. Enzymatic systems require optimum conditions themselves, therefore are not always applicable to industrial processes. Other bioprocesses include large scale synthesis in the pharmaceuticals industry, in which a control on the formation and consumption of key analytes or reactants such as glucose is required. A number of enzymatic glucose sensors have been developed and used in monitoring fermentation processes [221--224], yet non-enzymatic electrodes have yet to be practically applied.

7.2. Quality control

In the food industry quality control of food and drink products is essential, and measuring the quantity of various sugars to validate the quality, maturity, authenticity and nutritional value of the product is of considerable importance. Unlike in-situ environments such as inside a fermentor or reactor, analytical assessment of final products can involve secondary separation stages to enhance selectivity. The use of flow injection analysis, high pressure liquid chromatography and capillary electrophoresis is common in the food industry, yet a responsive, highly sensitive sensor is essential none the less. In Table 2 non-enzymatic electrodes have been applied in collaboration with these stages, and the considerably lower limits of detection demonstrate the high sensitivity of the electrodes towards the analyte [225--230]. Chromatographic stages are not always necessary however. Nonenzymatic glucose electrodes have been used to discriminatively determine aldose type sugars (e.g. glucose, xylose and galactose) versus ketose type sugars (e.g. fructose and sorbose) [96,212].

7.3. Fuel cells

In the controlled environment of a fuel cell, that is not restrained by the problems associated with physiological conditions, non-enzymatic glucose fuel cells are understandably under extensive investigation. As climate change and environmental issues take scientific precedence in the last decade, renewable power sources that are carbon neutral are highly sought, and as glucose is readily harvested in natural sugars and produced as a by-product to photosynthesis, it is an ideal fuel source to replace fossil fuels. Furthermore, the desire to fabricate a biologically powered artificial organ such as a heart or pacemaker [62,231--235] has been as great as to develop a glucose sensor. Glucose, or indeed other saccharide fuel cells, may therefore one day replace batteries in portable devices to engines in vehicles.

In comparison to other fuel cells the lure of a glucose based system is the potential to derive power from a degradable biomass, that is non toxic, non flammable and readily and renewably available, thus obtaining a high energy output from a sustainable source. Furthermore, glucose theoretically has a very high energy density, with the capacity to release up to 24 electrons per glucose

molecule on the basis of complete oxidation to CO_2 and H_2O [236]. The complete oxidation in a fuel cell would theoretically yield the processes in equation 7 at the anode and cathode, occurring at a formal potential of 1.23 V and releasing 2870 kJ mol^{-1} in energy [237].

$$\begin{aligned} &\text{Anode} && C_6H_{12}O_6 + 24OH^- \rightarrow 6CO_2 + 18H_2O + 24\,e^- \\ &\text{Cathode} && 6O_2 + 12H_2O + 24\,e^- \rightarrow 24OH^- \\ &\text{Overall} && C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 18H_2O \end{aligned} \qquad (7)$$

The potential energy stored in glucose is substantial, yet enzymatic, and most non-enzymatic electrodes only oxidize glucose to the glucolactone, a mere two electron process that yields 250 kJ of energy [237]. This will always be the case at enzymatic systems, yet non-enzymatic catalysts have the potential to fully oxidize glucose. It has already been claimed by Larew et al [50] that glucose may be oxidized beyond glucolactone on a gold electrode, depending on the electrode potential and on the glucose concentration. Mass spectrometry has also been used and shown signs of CO_2 formation at potentials well into the oxide region for gold [63], and Kokoh et al. used HPLC to analyze the products, and observed not only glucolactone and gluconic acid, but products derivative of further glucose oxidized [238]. Applying a high potential would defeat the object of a fuel cell, yet considering the significant decrease in operating potential for the initial glucose oxidation at modified gold electrodes [81,96,97], the potential required to fully or more substantially oxidize glucose may be shifted also. Such an electrode was reported by Jin and Taniguchi [239] in 2007 using a silver modified gold electrode.

A number of non-enzymatic glucose fuel cells have now been reported on a range of electrode materials including silver modified gold [239], silver microfibers [240] platinum [241] and very recently carbonized nanofibres [242]. The application of non-enzymatic glucose electrocatalysts towards fuel cells is an extensive topic in itself, and a number of comprehensive reviews on the subject have been recently published [236,237].

8. CONCLUSION

It is evident that the field of non-enzymatic glucose sensing electrodes is developing rapidly given the vast number of publications observed in the past 10 years. The surge in interest and improved success no doubt coincides with the development of nanomaterials, allowing for higher surface areas, improved glucose oxidation kinetics, and better selectivity. However, it is also evident that much of the research surrounding non-enzymatic glucose sensors is becoming repetitive and greatly lacks innovative approaches beyond designing new nanomaterials. The fabrication of a variety of nanostructures and modification of relatively new electrode materials, though aesthetically pleasing in the publication, are posing no significant improvement relative to enzymatic electrode design. The sophisticated and innovative ‘wired’ enzyme design developed by Heller et al [19,20,243] over the

past 20 years epitomises the idea of a new and exciting electrochemical approach, and it is a comparable advancement that is missing from non-enzymatic research. This advancement may depend on research into new carbon based materials, presently a very hot topic however. The technology involving carbon nanotubes, graphene, carbon nanofibres and doped diamond is certain to rapidly develop in the near future, possibly leading to a useful non-enzymatic glucose sensor.

Although there is a clearer understanding of the mechanisms of direct glucose oxidation at metals and carbon electrodes they remain uncertain, with frequent contradictions observed in the literature. As such a universally agreed mechanism for non-enzymatic glucose oxidation remains unresolved, and as such real modification of electrodes can not proceed effectively without this basic understanding. Non-enzymatic glucose electrodes offer high sensitivity, the possibility of long term stability, a resistance to thermal implications, and a low cost, simple and reproducible fabrication method, and as such they require a renewed approach to their design and development. However, they have yet to achieve all the requirements of a viable blood glucose sensor as stipulated in Tables 6 and 7, and have some limitations to overcome before they supersede enzyme technology, though it would seem the goal of non-enzymatic glucose sensing is close to being realized.

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