Iranian Journal of Analytical Chemistry
Volume 3, Issue 1, March 2016 (1-26)

مجله ایرانی شیمی تجزیه
دوره ۳، شماره ۱، فروردین ماه ۱۳۹۵ (۲۶-۱)

مروری بر اصول و پیشرفت‌ها در بیوحسگرهای اوره

اشرف سلمانی پور*[۱و]، علیرضا محدثی[۲]، محمدعلی کریمی[۲]، محمدعلی طاهر[۱]، شبنم شفیعی[۲]

۱. بخش شیمی، دانشگاه شهید باهنر کرمان، کرمان، ایران
۲. گروه شیمی، دانشگاه پیام نور، صندوق پستی ۳۶۹۷- ۱۹۳۹۵، تهران، ایران

تاریخ دریافت: ۱۱ بهمن ۱۳۹۳ تاریخ پذیرش: ۲٤ اسفند ۱۳۹۳

A Review on Fundamentals and Progress in Urea Biosensors

Ashraf Salmanipour*[,1], Mohammad Ali Karimi[2], Alireza Mohadesi[2], Mohammad Ali Taher[1], Shabnam Shafiei[2]

1. Department of Chemistry, Shahid Bahonar University of Kerman, Kerman, I.R. IRAN
2. Department of Chemistry, Payame Noor University (PNU), P.O. Box 19395-3697, Tehran, Iran

Received: 31 January 2015 **Accepted:** 15 March 2015

چکیده

علاقه‌مندی به آنالیز اوره در نمونه‌های مختلف بالینی، محیطی و صنعتی در چند دهه گذشته مبنای بسیاری از تحقیقات در زمینه ساخت بیوحسگرهای اوره بوده است. این فعالیت‌ها شامل استفاده از گستره وسیعی از مواد برای تثبیت آنزیم اوره آز و استفاده از آن در تشخیص اوره بوده است. این مواد شامل موارد زیر است: پلیمرهای هادی و غیر هادی، رنگ‌های ردوکس، اکسیدها، خاک‌ها، زئولیت‌ها، سل-ژل، خمیرهای کربنی، بسترهای فعال‌شده اپوکسی و نانومواد. اندازه‌گیری اوره در سطح چنین بیوحسگرهایی با استفاده از مدهای مختلف اندازه‌گیری از جمله پتانسیومتری، آمپرومتری، هدایت‌سنجی، حرارت‌سنجی، نوری، مانومتری و پیزوالکتریک گزارش شده است. در مقاله حاضر کوشش‌های تحقیقاتی در چند دهه اخیر بر روی ساخت و کاربرد بیوحسگرهای اوره برای آنالیزها اوره در نمونه‌های مختلف مرور شده است.

واژه‌های کلیدی

اوره آز؛ بیوحسگر اوره؛ نمونه‌های بالینی؛ تثبیت؛ تبدیل.

Abstract

Considerable interest in the analysis of urea in clinical, environmental and industrial samples has generated diverse research activities in the fabrication of urea biosensors over the past decades. These activities have been directed towards the use of wide ranging materials, including conducting polymers, non-conducting polymers, redox dyes, redox polymers, oxides, clays, zeolite, sol gel, carbon pastes, epoxy activated support and nanomaterials, for the immobilisation of urease and its use for the detection of urea. Many of these activities have also employed various modes of transduction, including amperometric, potentiometric, conductometric, optical, manometric, thermal and piezoelectric detection, for reliable biosensing of urea. This article reviews the various research efforts that have been carried out over the past decades on the construction and utilisation of urea biosensors for urea analysis in various samples.

Keywords

Urease; Urea Biosensors; Clinical Samples; Immobilisation; Transduction.

1. INTRODUCTION

The interest in the reliable determination of urea has increased considerably over the past four decades because of the growing awareness of its clinical, environmental and industrial signify-cance. In humans and animals, urea is formed exclusively in the liver and it is transported from here through the bloodstream to the kidneys where it is excreted into the urine [1]. It is the predominant final metabolite of nitrogenous compounds in mammals, accounting for 80– 90% of nitrogen excretion in humans. For this reason the monitoring of urea concentrations in human blood or urine has become an important diagnostic approach for assessing the condition of the kidneys and associated health effects. Patients who suffer from kidney disorder often experience the accumulation of urea in the blood. Normal blood urea concentration in human ranges from 1.3 to 3.5 mM [2-3], but the serum urea concentrations in people with high and chronic kidney diseases can be up to 12-15 mM and 5–7

*Corresponding Author: a_salmanipour_k@yahoo.com

mM, respectively [4-5].
Regular dialysis treatment may be required once or twice a week to reduce blood urea concentration and this can take about 3 to 5 hrs. The reliable optimisation of the time required to reduce blood urea concentration during the dialysis process can only be achieved if a suitable continuous monitoring method is available [6]. Such a method will be beneficial for avoiding unnecessary prolongment of the dialysis process and, hence minimising associated costs. Apart from its clinical significance, other area where the reliable determination of urea is of interest include the analysis of various environmental, industrial, cosmetic, agricultural, and food products [7-8]. Various samples, such as milk [9-11], wine [12], fertilizers [13], coastal waters [14-16], and soils [17], have been analysed for urea. Table 1 summarizes the urea concentration ranges found in some of these environmental and biological samples.

Table 1. Typical urea concentrations in some clinical and environmental samples.

Sample	Concentration range (mM)	Ref.
Urine	Up to 400	[1]
Blood (normal range)	1.3-3.5	[2,4]
Blood (pathophysiological range)	Up to 100	[3]
Oceanic waters	$<5\times10^{-4}$	[1]
Milk (normal range)	3-5	[11]
Estuarine/Coastal waters	1×10^{-5}-2.4×10^{-2}	[13,16]

The development of analytical methods that can reliably determine urea at these concentration ranges has been the subject of interest for several research groups around the world for almost four decades [1-18]. In general, the methods that have been reported to date can be classified as either *direct* or *indirect*. Indirect methods are based on the use of the enzyme, urease, to quantify urea concentrations indirectly from the amount of NH_3, NH_4^+, CO_2, HCO_3^- or the associated pH change generated by the catalytic hydrolysis of urea as illustrated by the following reaction [1,16]:

$$(NH_2)_2CO + 2H_2O \xrightarrow{\text{Urease}} 2NH_3 + H_2CO_3 \quad (1)$$

On the other hand, direct methods often involve the generation of a coloured product from the reaction of urea with chemical reagents without the need for prior degradation. However this category is often expanded to include miscellaneous methods that do not involve enzymatic degradation or colorimetric measurement, such as manometric, refractive index and direct infrared (IR) or ultraviolet (UV) absorbance measurements [1,18]. In recent years, most of the indirect analytical methods for the determination of urea concentrations are based on the use of biosensors, which employ the urease-catalysed hydrolysis of urea and the detection of one of the catalytic products.
In this paper, we review the considerable research activities that have been undertaken in the past decades on the fabrication of reliable biosensors for the determination of urea concentrations in clinical, environmental and industrial samples. The review highlights the wide ranging approaches that have been employed for the fabrication of urea biosensors, including the use of various materials, such as conductive polymers, non-conductive polymers, redox dyes, redox polymers, oxides, clays, zeolites and sol-gels for this purpose. Recent and future trends towards the use of nanomaterials, such as nanoparticles, nanotubes, nanolayers, nanofibers, nanohybrids and nanocomposites, for fabrication of more sensitive and robust urea biosensors will also be highlighted.

2. BIOSENSING OF UREA VIA UREASE-CATALYSED HYDROLYSIS OF UREA

The underlying principle for most urea biosensor is based on the detection of a product(s) generated from urease-catalysed hydrolysis of urea. As illustrated in reaction (1), urease, a nickel-dependent metalloenzyme, catalyses the hydrolysis of urea to ammonia and carbonic acid. Both of these products are then further hydrolysed to generate ammonium and bicarbonate ions:

$$2NH_3 + 2H_2O \rightarrow 2NH_4^+ + 2OH^- \quad (2)$$

$$H_2CO_3 + H_2O \rightarrow H_3O^+ + HCO_3^- \quad (3)$$

Therefore, the overall hydrolysis reaction of urea results a net increase in pH, as illustrated below:

$$(NH_2)_2CO + 3H_2O \xrightarrow{\text{Ureas}} 2NH_4^+ + HCO_3^- + OH^- (4)$$

It has been reported [19-20] that the rate of urease-catalyzed hydrolysis of urea is 10^{14} times faster than that of an uncatalyzed reaction. Consequently, sufficient quantities of catalytic products are generated from this reaction for detection by a range of analytical methods. The detection of urea by this approach with various biosensors based on the use of different transduction systems will be highlighted in this review. However, it is worth noting at this stage that in practice, the free enzyme (urease) is usually immobilized on an inert matrix and can be used continuously for several analyses over a period of time [21].
The first urea biosensor was reported in 1969 by Guilbault et al. [22]. In that work they used a cation selective glass electrode which contained an enzyme (urease) layer to obtain a signal for urea based on the detection of the ammonium ion activity which resulted from the urease-catalyzed

hydrolysis. Since then, the use of urease as a biocatalyst for the development of urea biosensors has attracted considerable interest, and various types of urea biosensors have now been reported [2-6, 11, 21-165].

The general operating scheme of a urea biosensor is shown in Fig. 1.

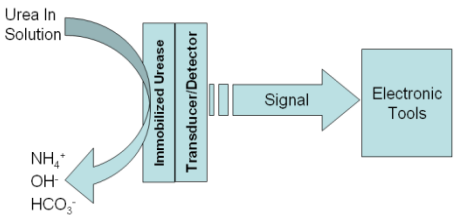

Fig. 1. Schematic illustration of the processes involved in the determination of urea with a biosensor.

Thus, the fabrication of a urea biosensor involves the initial immobilisation of urease onto a suitable membrane or support on which the hydrolysis of urea to ammonium and bicarbonate ions takes place [21]. The enzymatic product(s) is then detected by a suitable transducer. In this way, the detection of urea is achieved by the measurement either of carbon dioxide, ammonia, ammonium, carbonate, or bicarbonate. In addition, the increase in pH resulting from the catalytic products permits pH-based detection for the indirect determination of the urea concentration [23] (also see Table 2, third column).

In some cases multi-enzyme systems are employed where the resulting catalytic product is detected by another enzyme catalysed reaction. On such example is a multi enzyme system, where the ammonia derived from the urease-catalysed reaction was determined by reaction with α-ketoglutarate, catalysed by L-glutamic dehydrogenase as illustrated below [21]:

$$\mathrm{KG} + \mathrm{NAD(P)} + \mathrm{NH_4^+} \xrightarrow{\mathrm{GDH}} \mathrm{GL} + \mathrm{NAD(P)^+} + \mathrm{H_2O} \qquad (5)$$

where KG, GDH and GL, are ketoglutarate, L-glutamic dehydrogenase, and L-glutamate, respectively. In this reaction, NADH or NADPH is oxidized and its decrease in concentration is detected electrochemically or optically [21,24-25]. The main benefit of this approach is its ability to convert optically and electrochemically inactive products of urea hydrolysis to an electroactive and optical active compound (NADH).

Table 2 summarises the properties of various urea biosensors that have been reported in the literature between 1995 and 2013 and these are reviewed in more details below, with particular emphasis on enzyme immobilisation strategies and the choice of modes of transduction. In the following, we will more explain the highlighted reports based on immobilisition matrix and types of transducers.

3. IMMOBILISATION OF UREAS

The adequate immobilisation of urease onto or into a suitable support is of pivotal importance for the successful construction of a usable and reliable urea biosensor, as it provides the medium for (a) the catalytic hydrolysis of urea, (b) detection of the resulting enzymatic product(s) and (c) signal generation. To this end the three main requirements for the successful immobilisation of bioactive substances, as part of a strategy for fabrication of biosensors, are [27]: (i) adequate retention of the bioactive substance on/within support matrix, with little or no lost in activity; (ii) ability to achieve the desired catalytic reaction after the immobilisation; and (iii) achievement of improved selectivity and sensitivity for the analyte by the resulting biosensor. Consequently, a proper choice of adequate matrix or support for the immobilisation of urease is a very significant first step in the fabrication of urea biosensors. The adequacy of the immobilisation of the enzyme governs the ultimate reliability and performance of the resulting biosensor. The commonly used matrices for immobilisation of urease for the construction of urea biosensors are discussed below.

3.1. Conductive polymers

One of the areas which have generated much interest in the fabrication of urea biosensors is in the use of conducting polymers. These polymers provide suitable matrices for immobilisation of bioactive substances and can be used to enhance stability, speed, sensitivity and, as such, have gained increased use in medical diagnostics [26-29]. A number of immobilisation techniques, such as physical adsorption, electrochemical entrapment, crosslinking and covalent attachment based on ethyl-dimethylaminopropylcarbodiimide (EDC) and *N*-hydroxy-succinimide (NHS) coupling chemistry, have been used to achieve improved stability of the desired biomolecules within or on conducting polymers [28]. In particular, the incorporation of biomolecules into electrodeposited conducting polymeric films permit the localization of bioactive molecules on electrodes of any size or geometry and are very ideal for the fabrication of multi-analyte micro-amperometric biosensors [28-29].

Electrically conducting polymers are known to have considerable flexibility in chemical

structures which can be modified as required. By chemical modelling and synthesis, it is possible to modulate the required electronic and mechanical properties of conducting polymers.
Moreover, the polymer itself can be modified to bind protein molecules [26].

Table 2. Characteristies of various urea biosensors.

Immobilization Matrix	Transducer	Detected Compound	Preparation Time (~h)	Linear Range (mM)	Detection Limit (µM)	Sensitivity	Response time (min)	Life time (day)	Sample	Reference
Conductive polymers:										
PPy/PVS	V	NADH	0.5							21
PPy	C	Ions	0.5	0.05-5.8		140 [c]		25		34
PPy	A	NH_4^+	0.1	0.05-0.25		1.02 [a]	<4		Urine, Blood	41
PPy	A	NH_4^+	0.1	0.002-0.075	1		<4	14	Blood	43
PPy	A	NH_4^+	4	1.7-56		0.001 [a]	<3	3	Blood	44
Poly(3-aminopyropyl pyrrole-co-pyrrole)	A	pH	3	0.16-5.05	20	470 [a]	0.7	60		45
PPy	-P	pH	3	10-100		63 [b]				46
	-A			0.01-100		7.6 [a]	1			
PPy/Polyacrylamide	O	pH	0.7	60-1000		0.013 [d]	<2			60
PPy/Biotinylated phospholipid	C	Ions	3	Up to 120	5000					68
PPy/$NaHCO_3$	P	pH	0.2	0.1-300		32 [b]	<3			69
Functionalized PPy	P	pH	10	0.14-0.01	1400	93 [b]				70
PPy/Polyion complex	P	pH	12	0.3-30		110 [b]	<0.5			71
PPy/Polyion complex	P	pH	12	0.01-3		120 [b]	<0.6		serum	72
PPy/PVS	P		55	5-60	1600	12 [b]	1	40		73
Poly(3-aminopyropyl pyrrole-co-pyrrole)	P	pH	3	0.006-0.4		28 [b]	<1	60		74
PPy	P			0.006-0.4		28 [b]				75
Poly(pyrrole-pyridinium)/ Laponite	C	Ions	2	Up to 1.5	10	0.085 [c]		7		76
PANI/Nafion	A	NH_4^+	2	0.05-0.5	5	316 [a]				4
PANI/Nafion	A	NH_4^+		0.001-1	0.5	0.76 [a]	<2	21	Urine	23
PANI/Poly (carbamoylsulphonat)	A	NH_4^+		Up to 0.1	5	40 [a]				42
PANI/Carbon felt	P									85
1- PANI/PVA	P		16	10-100		41 [b]			Serum, Milk	86
2- PANI/Sol-gel			36		20	45 [b]				
PANI /Poly(n-butyl methacrilate)	C	Ions	2	0.33-2		0.7 [c]			Serum	87
Poly(3-cyclohexyl thiophen)	P	pH	70	0.001-0.004	40	55 [b]	0.9	90	Milk	88
Non-conductive polymers:										
Poly(carbamoylsulfonate) /Polyethylenimine	P	NH_4^+	25	0.072-21	20	52 [b]	<2	4	Blood	2
PVC/Palmitic acid	P	NH_4^+	24	0.01-10		40-54 [b]	<2	60	Blood	3
PVC-COOH/Nonactin	P	NH_4^+	38	0.5-8			<3	60	Human serum	5
BSA/Glycerol	F	pH	1	Up to 2		18 [b]	<3		Blood	32
Nylon nets	A	NADH		0.01-0.3	10	0.001 [a]			Human Saliva S.	51
PVC-Polyurethane	O	NH_4^+	3	0.1-10		0.03 [d]	<4	3	Serum	54
PVC/C60	Z	NH_4^+	70	0.1-100	0.1	15 [g]				66
PVC-COOH/Nonactin	P	NH_4^+	38	0.1-70		50 [b]	0.25	49		67
Chitosan	P	NH_4^+	15	0.1-10		56 [b]	<2	60	Serum	92
Polyethylenimine	P	pH	12	3.1-31.6		7-25 [b]	<0.5			93
PVC-	P	NH_4^+	38	1.6-5			0.25		Hemodialysis	95

COOH/Nonactin									fluids	
PVC-COOH/Nonactin	P	NH_4^+	38	2-16		39 [b]	2.4		Hemodialysis fluids	96
PVC-COOH	O	pH	15	0.3-100		0.003 [d]	<3	21		97
PVC-COOH/n-tridodecylamine	P	pH	15	1-13		40 [b]	4	30	Urine, Cosmetic and Pharmaceuti-cal S.	98
PVC/Polyurethane	O	NH_4^+	30	0.08-2		55 [d]	0.3			99
Polyvinylpyrrolidone	P	NH_4^+		0.5-2.5			20			100
Poly(N-vinylcarbazol)/Stearic acid	P	NH_4^+	0.5	0.5-93	500	10 [b]	2	35		101
Poly(N-Vinyl pyrrolidone)	F	pH	0.2	0.05-20		38 [b]	<10	40	Serum	102
PVA-Styrylpyridinium (SbQ)	F	pH	5	0.8-13		132 [b]	<2	99		103
Nylon nets	Z	NH_3	17	0.01-3		0.33 [f]	3	28	Serum	104
Eupergit C	C	Ions	72	3.5-35				90	Dialysis fluids	123
PVC-BSA	P	NH_4^+	8	1-100		30 [b]	<1		Dialysis fluids	124
Gelatin	P	pH	25	2.5-23		67 [b]	2	80	Serum	125
PVC-COOH/Nonactin	P	NH_4^+	38	1-15		21 [b]	<2	30	Human serum	126
PVC/nonactin	P	NH_4^+		0.01-1		59 [b]	0.2	30		127
Poly-o-phenylenediamine	P		4	0.01-1		15 [b]		35		135
PVC	P	NH_4^+	30	0.02-1		40 [b]	1.5	60		136
Clays, Zeolites and oxides:										
Controlled porous glass	C	Ions	17	5-50			5	350	Blood	6
Clinoptilolite/Silopre ne	F	NH_4^+		0.03-0.5	30	15 [b]	5			31
1- [Zn-Al] LDH	F	pH	94	0.04-1.37	3.5	37 [b]	<0.2			107
2- Laponite				0.005-0.5	2	110 [b]	<4			
[Zn-Al] LDH	F	pH	36	3-100		19 [b]				109
[Zn-Al] LDH	-P	pH	94	0.56-5.6		21 [b]				137
	-M			3-110		0.002				
Al_2O_3	P	NH_3	24	0.03-14	10	54 [b]	<4	90	Urine	110
1- Controlled porous glass	C	Ions	37	Up to 150	0.5	53 [e]	5	310	Serum	111
2- Silica gel				Up to 150	0.5	51 [e]	5	310		
RuO_2/graphite	P	pH	6	1-31		53 [b]	2		Urine, Serum	112
RuO_2/graphite	P	pH	6	6-17.5		30 [b]			Hemodialysis fluids	113
SiO_2/Al_2O_3	P	pH	1	Up to 15		13.3 [e]				138
Silicalite/Zeolite Beta	C	Ions	24	Up to 6		11.6 [c]	1.3	7		142
Redox polymers and dyes:										
Poly(venylferroceniu m)	P	pH	20	0.05-100	5	13 [b]				105
Poly(venylferroceniu m)	A	pH	20	0.001-0.25	1	2.4 [a]	1	29		47
Hematein/Graphite powder	A	pH		0.025-0.35		0.199 [a]	<3		Urine	48
1-Lauryl	-A	pH	0.3	0.002-0.75	2	0.015 [a]	<5	30		49
gallate/Graphite	-A	pH	0.5	0.01-0.25	10	0.0001 [a]	<0.5	30		
2-Methylene blue										
Polytoluidine Blue	A	pH	0.2	0-0.8	20	0.98 [a]	<0.5			50
PB/4-(pyrrolyl)-benzoic acid	O	pH	48	2-16				60	Hemodialysis fluids	55

PB/4-(pyrrolyl)-benzoic acid	O	pH	48	3-100		0.002 [d]		60	Urine, Cosmetic S.	56
PB/4-(pyrrolyl)-benzoic acid	O	pH	48	0.2-100	100	0.119 [d]		60		57
Nickel hexacyanoferrate	A	NH_4^+	0.4	0.01-1		314 [a]	1	45		106
Poly(glycidyl methacrylate-co-vinylferrocene)	A	pH	75	0.1-4	60	0.0003 [a]	0.05			144
Sol-gel network:										
Sol-gel (TMOS)	C	Ions	24	0.2-50	200	8.2 [c]	4	21	Urine	36
Sol-gel (TMOS)	C	Ions	12	0.03-30	30	204 [c]	8	25	Serum	37
Sol-gel (TMOS)	O	pH	5	0.1-100	2.5	0.06 [d]	10		Serum	58
Sol-gel (TEOS)	O	pH	170	3.5-9		0.07 [d]	5			59
Sol-gel (APTES)	Z	pH	2	0.07-5			3			65
Sol-gel (APMES)	C	Ions	4	0.01-5			1.5			115
Nanomaterils:										
PANI nanofiber/ Pt nanoflower	A	NH_3	8	Up to 20	10	0.116 [a]	3			81
PAH/PSS nanofilms	O	pH	2	0.1-60			8		Dialysate samples	149
Gold NPs / polyester	A		60	0.01-35		0.007 [a]	0.05	3000	Blood serum, Urine	148
Nano porous alumina	Z	Ions		0.0005-3	0.2		0.5	30	Urine	147
Gold NPs / poly(allylamine)	V	Ions	3	Up to 3	2	107 [c]	3			156
Fe_3O_4 NPs	P	pH	5	0.01-0.3	0.01		1			154
CNT-PPy	M			0.0006-1	0.6		0.5	30		155
ZnO nanorods	A		10	1-20	130	0.4 [a]	0.05			152
Rhodium NPs / chitosan	A		50	1.6-8.2	500	3.1 [a]		10		151
CNT / silica matrix	P		12	0.002-1.07		23 [b]	0.3	60		150
NiO NPs	CV		27	0.83-16.6		21.3 [a]	0.1	14	Human serum	161
TiO_2-ZrO_2 nanocomposite	CV	NADH	12	0.8-16.6	440	2.74 [k]	0.16			159
Poly (glycidylmethacrylat) -grafted Iron oxide NPs	P		149	0.25-5	50	20 [e]	0.13	60	Blood, Urine	160
PANI-CNT	A	Ions	1	4-10		4 [a]	0.2	24		157
Silica NPs	A			0.3-4.5		2.05 [a]				158
Other matrices and approach:										
Enzyme is in Solution	N	CO_2					5		Milk	11
Carbon paste	A	NADH	3	0.02-0.2	5	500 [a]		15	Blood	24
Immobilon-AV membrane	A	NADH	3	0.02-0.2	5	50 [a]	3	15	Fertilizers	25
Immobilon-AV membrane	P	NH_4^+		0.5-30		59 [b]	1	30	Dialysis fluid	117
Si_3N_4	F	pH	15	0.3-10		66 [b]	1.2	35	Blood, Dialysis fluid	118
No report	L	HCO_3^-	20	0.004-0.4	2	7 [h]	<5	5	Urine	119
Poros 20 EP	T	Heat	13	1-15	0.5	2.5 [j]			Bovine serum	120
Agarose beads	T	Heat	15	1-25		1.2 [i]				121
Agarose beads	T	Heat	15	0.5-20		2.6 [i]	0.25			122
Unreported	F	pH		0-200						165

Units are (a) µA mM^{-1}, (b) mV per decade, (c) µS mM^{-1}, (d) Abs. mM^{-1}, (e) mV mM^{-1}, (f) KHz mM^{-1}, (g) Hz per decade, (h) I mM^{-1}, (i) °C M^{-1} (j) mJ mM^{-1} (k) µA $[\log mM]^{-1}$. PB=Prussian blue, PPy=polypyrrole, PANI=polyaniline, PVA=polyvinyl alcohol, PVS=polyvinyl sulphonate, PVC=polyvinyl chloride, BSA=Bovine serum albumin, CNT=Carbon nanotubes, NPs=nanoparticles. Transducers are: A=Amperometry, P=Potentiometry, O=Optical, T=Thermal, F=ISFET, L=Chemiluminesence, Z=Piezoelectric, C=Conductometry, N=Manometry, M=Impedometry, V=Voltammetry.

Another advantage offered by conducting polymers is that the electrochemical synthesis allows direct deposition of a polymer on the electrode surface, while simultaneously trapping the bioactive molecules [26]. It is thus possible to control the spatial distribution of the immobilised enzymes, the film thickness and modulation of enzyme activity by changing the state of the polymer. Several conducting polymer-based urea biosensors have been fabricated by entrapment of urease within electropolymerized polypyrrole (PPy) films [21,34,41,43-46,60,68-76], polyaniline (PANI) films [4,23,42,85-87] and polythiophene films [88]. Some of the significant urea biosensors reported in this area are discussed below.

3.1.1 Polypyyrole-based urea biosensors

In one of the earlier work on the use of polypyrrole for urea biosensors, Adeloju et al. [41] fabricated an amperometric biosensor for urea by utilizing an electropolymerized conductive PPy-urease film and achieved a linear concentration range between 50 and 250 µM. The PPy-urease film was prepared from an aqueous solution containing pyrrole and urease, without the addition of a supporting electrolyte. The pI of urease is 4.9 and its molecules are anionic at pH > 5. Thus, urease can be entrapped easily into polycationic matrix of conductive polymers during electropolymerization [88-90]. Mechanistically, the biosensor functions by the interaction of urea with the PPy-urease film and the resulting catalytic product(s) causes a change in conductivity at the electrode surface. Consequently, this interaction results in a shift in the overall cell potential to a more positive direction which enables the oxidation of the PPy and, therefore, results in a current change [41]. Adeloju et al. [43] later improved the achievable detection limit to ~2 µM by utilization of a pulse-amperometric technique for detection of urea and successfully utilised the biosensor for the quantification of urea in blood samples, as illustrated in Fig. 2. Komaba et al. [69] reported on another urea biosensor, prepared by electropolymerization of pyrrole in an aqueous solution which contained pyrrole, nucleophilic electrolyte and urease [69]. The use of a nucleophilic electrolyte as a supporting electrolyte resulted in the formation of an electroinactive PPy due to its promotion of the overoxidation of PPy as it is formed. However, only a small amount of urease could be immobilised in the overoxidised PPy film by the electropolymerisation process. Consequently, the resulting urea potentiometric biosensor was only useful for the measurement of urea within a linear concentration range of 0.1 – 300 mM. Komana et al. [91] also reported on the use of a composite film of electroinactive PPy which contained urease modified with water soluble polyanion (polyacrylic acid), as a means of increasing the amount of immobilised urease.

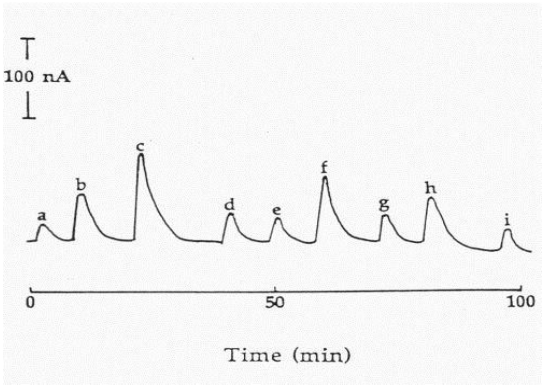

Fig. 2. Application of PPy-urease biosensor to the flow injection amperometric analysis of urea in blood. Urea concentrations: (a) 0.01, (b), 0.1, and (c) 1 mM. Responses (d) to (i) are for blood samples. Reproduced from [43].

The sensitivity of this composite electrode increased and gave a slope of 53 mV decade^{-1}. At about the same time, Osaka et al. [71] proposed a two-step process for fabricating a composite film of electroinactice PPy/polyion complex including urease [71]. As a first step, a polyion complex (polyacrylate as polyanion and poly-L-iysine hydrobromide as polycation) which includes urease was pre-coated onto a platinum electrode and then followed by the electrodeposition of a non-conductive PPy film [71]. This insoluble water polyion improved sensitivity of the resulting potentiometric biosensor to urea and achieved a sensitivity of 110 mV decade^{-1}. The biosensor was subsequently applied to flow injection analysis of urea, enabling faster and easier determination of urea [72]. Under optimized analytical conditions, 10 µM to 3 mM urea could be measured with the biosensor at a higher sensitivity of 120 mV decade^{-1}.

Trojanowicz et al. [44] constructed a urea biosensor by immobilisation of urease on an ammonia sensitive PPy film with the aid of glutaraldehyde (GLA) as an immobilisation agent [44]. This GLA immobilised urease was used for the amperometric biosensing of urea within a concentration range of 2-50 mM with a sensitivity of 1 nA mM^{-1} and was successfully applied to the determination of urea in human blood serum samples.

Ghourchian et al. [34] reported on the use of covalent binding for the immobilisation of urease on the surface of a platinum electrode coated with polypyrrole. In this case two different procedures

were employed: (i) chemical binding in an albumin-glutaraldehyde film, and (ii) entrapment by electropolymerisation of pyrrole. They reported that more reproducible conductometric responses and a lower detection limit of 49 µM were obtained with the urea biosensor produced by covalent binding. Also, a linear concentration range of 49 µM to 5.8 mM was obtained.

Gambhir et al. [73] also constructed a potentiometric urea biosensor by covalent bonding of urease on PPy microspheres and then chemically linked them to conducting PPy-polyvinyl sulfonate films. The use of PPy microspheres is known to enhance the loading of an enzyme, since the surface area available for its immobilisation can be increased many times as compared to smooth surfaces. Potentiometric measurements on this conducting polymer biosensor with the aid of an ammonium ion analyzer enabled the determination of urea concentrations within a linear concentration range of 5 to 60 mM.

In another work, Hernandez et al. [70] also immobilised urease by covalent bonding to amine groups on a functionalized PPy film via a carbodiimide reaction. The resulting film gave a good response to urea, and enabled the achievement of a detection limit of 140 µM. The main advantage of this urea biosensor was its improved stability which is associated with its ability to minimise enzyme loss through leaching.

Rajesh et al. [45,74-75] electrochemically prepared a poly(amino propyl pyrrole-co-pyrrole) doped with large size dopant anion (para-toluene sulfonate) as an immobilisation platform for obtaining high enzyme loading at the polymer surface. The incorporation of large size dopant into PPy film during electropolymerisation makes PPy film more porous to the facile immobilisation of enzyme. Also, free amine functional groups on the polymer were used for covalent bonding with urease to improve lifetime stability of the enzyme. This film was employed for potentiometric [74-75] and amperometric [45] determination of urea with linear ranges from 6 to 400 µM and 0.16 to 5.05 mM, respectively. The response time was 40 s and 80% of the enzyme activity was retained for 2 months.

Marcos et al. [60] used acrylamide to photoimmobilized urease on a chemically polymerized PPy film and employed the resulting film for optical detection of urea. The main advantage of this sensor is that no indicator dye or pH indicator was needed because PPy itself acts as the support and the indicator. However, the optical biosensor was not very sensitive and urea was only detected within a linear concentration range of 0.06 to 1 M of urea. Also, the lifetime of the optical biosensor was short, lasting only 1 week.

In another study, Senillou et al. [76] coated urease onto a laponite clay gel and crosslinked the enzyme with glutaraldehyde. The latter coating was adsorbed onto an electrochemically generated poly(pyrrole–pyridinium) film on an interdigitated microelectrode array. This microbiosensor achieved a detection limit of 10 µM and can determine urea concentrations up to 5 mM. The use of this inorganic-organic composite was useful for overcoming the reduction in enzyme activity, attributed to the hydrophobic nature of PPy as an organic host polymer.

The conductance and electrostriction of bilayer lipid membranes (BLMs) supported on stainless steel and on conducting polymer PPy (PPy) were investigated by Hianik et al. [68]. A urea mini sensor based on BLMs supported on a PPy layer was developed. Avidin-biotin technology was applied for the immobilisation of urease on BLMs and a good sensitivity was achieved with this device for the determination of urea. The detection limit of the mini biosensor was approximately 5 mM urea. This was not as good as those achieved for urea biosensor based on PPy deposited onto a platinum electrode [44].

More recently, Jin et al. [46] patterned platinum thin-films on nano-prous silicon layers by using platinum RF sputtering. Then urease was immobilised into PPy electropolymerised on this thin film for biosensing of urea. Porous silicon layers provide large electrode surface area which enhanced the sensitivity of the biosensor. The amperometric detection of urea with this biosensor was more sensitive and gave a wider linear range (0.01 – 100 mM) than by potentiometric detection (10-100 mM).

In another approach, Gambhir et al. [21] co-immobilised urease and L-glutame dehydrogenase (GDH) into an electrochemical synthesised PPy-polyvinyl sulfonate films by both physical adsorption and electrochemical entrapment methods. They also carried out detailed investigation of influence of pH and investigated the behaviour of the films by Fourier transform infrared spectroscopy, cyclic voltammetry, and scanning electron microscopy. The action of this bienzyme system in presence of urea decreased the electrochemically detectable NADH concentration.

3.1.2 Polyaniline-based urea biosensors

Another conducting polymer that has attracted some interest for the fabrication of urea biosensors is polyaniline (PANI). Luo and Do [4] and Cho and Huang [23] fabricated amperometric urea biosensors by cross-linking urease onto a

PANI-Nafion composite electrode. The presence of Nafion in the PANI matrix enabled the detection of ammonium ions based on the doping/undoping NH_4^+ into the polymer matrix. The scheme for the sensing mechanism of urea on the PANi(urease)-Nafion® composite electrode is illustrated in Fig. 3. When urea in the bulk solution was transferred to the enzymatic active site located in the PANi-Nafion® composite film, the ammonium ion generated could be doped into the PANi-Nafion® composite film membrane, and then the composite film was electrochemically reduced from its oxidised state. These biosensors achieved excellent detection limits of 5 µM [4] and 0.5 µM [23], respectively. Also, the shielding effect of the layer of urease immobilised in bovine serum albumin (BSA) was useful for separation from other ions.

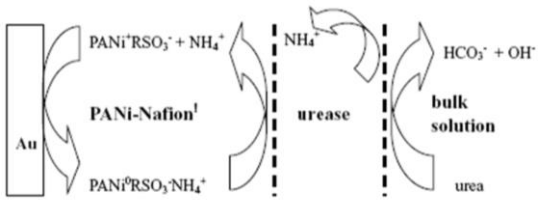

Fig. 3. Scheme of the sensing urea on urease/PANi-Nafon®/Au/ceramic plate composite electrodes [4,23].

Strehlitz et al. [42] prepared a PANI-modified screen printed platinum electrode from aniline and sulphuric acid solution and then immobilised urease on the electrode in a poly(carbamyl sulphonate) hydrogel. The gel was included to enable the achievement of a high operational stability for the enzyme. Amperometric determination of ammonia with this biosensor gave a detection limit of 5 µM and a linear concentration range of 5 – 100 µM. The operational stability was maintained for 500 consecutive measurements in a FIA-system, reaching 50% of the initial activity, as shown in Fig. 4.

In another study, Pandey et al. [86] incorporated tetraphenylborate, a known non-specific relatively hydrophobic ion exchanger, within a PANI film. The resulting polymer was pH-sensitive, retaining reproducibility for more than 9 months without loss of potentiometric response, while being useful in both non-aqueous and aqueous medium. The resulting pH sensor was exploited for the construction of solid-state urea biosensor by immobilising urease within either poly vinyl alcohol (PVA) or a sol-gel network PANI modified electrode. This urea biosensor gave a detection limit of 20 µM and was successfully used for the determination of urea in serum and milk.

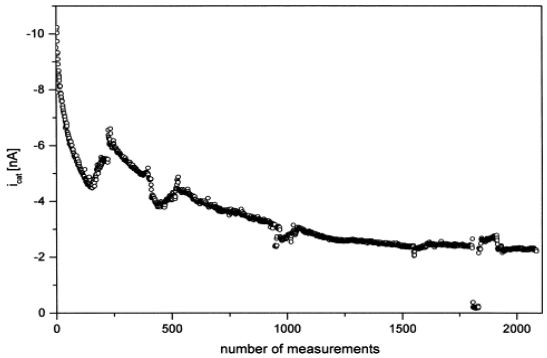

Fig. 4. Operation stability of a PANI-urease biosensor used in a FIA mode. Measurements were based on consecutive injections of 1 mM urea every 4 min. Reproduced from [42].

Castillo-Ortega et al. [87] also prepared homogeneous electroconductive films of PANI-poly(n-butyl methacrylate) composites with a surfactant (PVME or PVEE) by using a casting method. Poly(vinyl methyl ether), PVME, or poly(vinyl ethyl ether), PVEE, were used as dispersant to improve morphological and mechanical properties of PANI composite. The composites with PVME were tested as a NH_3 conductometric sensor, and gave good analytical performance. Also, urease was immobilized on this electroconductive film for biosensing of urea and a linear concentration range was between 0.33 and 2 mM. This detection range was adequate for the analysis of urea in serum samples. Uchiyama et al. [85] also immobilised urease into a PANI matrix which was electrodeposited on a carbon felt electrode. Urease activity was varied by changing the electrode potential of the porous carbon felt immobilised urease. It was found that the loss of urease activity by oxidation is recovered by both chemical and electrochemical reduction.

Another conducting polymer that has gained limited use for fabrication of urea biosensors is polythiophene. However, in one study Pandey et al. [88] developed a solid state pH sensor by using neutral poly(3-cyclihexyl thiophene) deposited on a platinum disk electrode [88]. Urease was then adsorbed onto this electrode before forming an organically modified sol-gel layer over the urease-adsorbed polymer modified electrode. A detection limit of 40 µM was achieved with the resulting biosensor, with a relatively high sensitivity of 55 mV decade^{-1}. The biosensor was found to be stable for 3 months when stored at 4 °C under dry condition.

3.2. Non conductive polymers

Many non-conductive organic polymers have also been used with various immobilisation methods to

construct a range of urea biosensors. In one of these studies Magalhaes and Machado [92] fabricate potentiometric urea biosensors by immobilising urease into chitosan membranes coupled to all-solid-state nonactin ammonium ion selective electrodes to. They compared the response characteristics of the biosensors prepared with four different immobilisation methods for the incorporation of urease into the chitosan membranes: (a) adsorption; (b) adsorption followed by reticulation with dilute aqueous glutaraldehyde solution; (c) activation with glutaraldehyde followed by contact with the enzyme solution; and (d) activation with glutaraldehyde, contact with the enzyme solution and reduction of the Schiff base with sodium borohydride. They found that the potentiometric urea biosensor prepared by method (b) gave the best response characteristics and was able to detect urea within a linear concentration range of 0.1 to 10 mM, with a sensitivity of 56 mV decade^{-1}, response time of about 1 min and life time of 2 months.

Lakard et al. [93] used a glass-sealed platinum electrode coated with a thin polyethylenimine film which is sensitive to pH to construct a potentiometric pH sensor. They then used this electrode as a basis for constructing a potentiometric urea biosensor. They also compared the use of a number of immobilisation methods for the incorporation of urease onto the electrode and it was found the immobilisation of urease by crosslinking with glutaraldehyde has many advantages, compared with those achieved with other reagents. The GLA immobilised urease biosensor exhibited a short response time of 15-30s, sigmoidal responses for urea within a linear concentration range of 3.1 to 31.6 mM and a life time of 4 weeks.

Karakus et al. [3] also investigated four different immobilisation methods for immobilising urease onto a PVC ammonium membrane which contained palmitic acid and involve using nonactin as an ammonium-ionophore [3]. The resulting potentiometric urea biosensor gave a relatively good analytical characteristics and performance with a relatively high sensitivity of 40 to 54 mV decade^{-1}, a dynamic stability over 2 months with relatively small decrease in sensitivity and a response time of 1–2 minutes. The potentiometric biosensor was successfully used for rapid determination of urea concentrations in serum samples. There was a good correlation between the results obtained with this biosensor and a standard spectrophotometric method for the determination of urea in human serum samples.

Koncki et al. [67,94] constructed potentiometric urea biosensors by combining urease with ammonium-selective membranes. Carboxylated PVC (PVC-COOH) was used as a support polymer in ion-selective membrane and nonactin was applied as an ammonium selective ionophore. The carboxyl groups located on the membrane surface enabled covalent attachment of urease directly on the membrane, without the need for additional protective membrane. The biosensor was able to determine urea within a linear concentration range of 0.3 to 70 mM at a response time of <20s and has a dynamic stability of over 2 months without a decrease in sensitivity [67]. This biosensor was subsequently employed in a FIA system [5] and achieved a linear concentration range of 0.5 to 8 mM with a response time of ≤2 mins. In the FIA mode, the biosensor was used for fast and accurate human serum analysis. The results obtained were in good agreement with the reference values for the samples. Other advantages include the simplicity of the system and low costs of measurements. The biosensor was further used [95] in a simple flow-injection analysis system for monitoring of hemodialysis progress by potentiometric determination of dialysate urea nitrogen (DUN). Over 25 DUN determinations were achieved in an hour, within a linear concentration range of 1.6 to 5 mM DUN. The urea biosensor/FIA system was also successfully used [96] to evaluate clinical parameters of urea kinetic modeling: parameters KT/V, urea reduction ratio (URR), percentage removal of urea (PRU) and total urea removal (TUR) for intradialytic intervals and urea generation rate (G) and protein catabolic rate (PCRn) for interdialytic intervals. These biomedical parameters are used for describing the adequacy of hemodialysis therapy. In another study [98], n-tridodecylamine was composited as the hydrogen-ion selective ionophore with PVC-COOH as the support and urease was covalently attached to the membrane surface via carboxyl groups on PVC network. The resulting potentiometric biosensor was again successfully applied to the determination of urea in urine, cosmetic creams and pharmacutical samples. Koncki et al. [97] also used a lipophilic pH indicator, 9-(4-diethylamine-2-octadecyloxy-styryl)-acridine, with plasticised PVC-COOH for the preparation of a pH optode membrane. Urease was then covalently linked to the surface of the pH optode membrane to form a urea optical biosensor. The resulting biosensor was used for rapid determination of urea within a linear concentration range of 0.3 to 100 mM.

Kovacs et al. [99] also developed a planar wave-guide type optical urea sensor based on the detection of the ammonium ions liberated during

the catalytic reaction with an ion selective optode membrane which contained nonactin as ion selective ionophore and ETH 5294 chromoionophore in a thin plasticized PVC film. The sensor contained an ammonium sensitive, 1 mm thick optode layer and a second covering layer made of polyurethane. Urease was immobilised by cross-linking with glutaraldehyde on the surface of the secondary layer. The biosensor achieved a linear concentration range between 0.08 and 2 mM, as well as a fast response time which enabled the analysis of about 30 samples per hour.

The electrospinning of a urease solution with polyvinylpyrrolidone (PVP) was used to develop a urea biosensor by Sawicka et al. [100]. The resulting material was tested for the detection of urea. In the process of electrospinning, as shown in Fig. 5, a mixture of polymer and enzyme solutions are injected from a small nozzle under the influence of an electric field as high as 30 kV. The build up of electrostatic charges on the surface of a liquid droplet induces the formation of a jet. The jet is subsequently stretched to form a continuous fibre. Before it reaches the concurrently charged collecting screen the solvent evaporates or solidifies. The fibers are collected on a conductor surface and form non-woven mats that are characterized by high surface areas and relatively small pore sizes. This improves the adsorption properties and enhances the sensitivity of the biosensor. Urease-PVP nanofibers prepared by this approach achieved a linear concentration range of 0.5 to 2.5 mM, and a response time between 10 and 30 s.

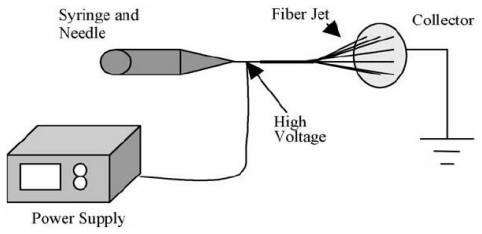

Fig. 5. Electrospining setup used for urease immobilization. Reproduced from [100].

In another interesting study, urease was immobilised in mixed monolayers of poly(N-vinyl carbazole) and stearic acid formed at an air/water interface [101]. The monolayers were transferred onto indium-tin-oxide (ITO) coated glass plates using Langmuir/Blodgett film deposition technique. This technique for monolayer deposition is known to facilitate the desired orientation of a biomolecule. Only a few investigations have been reported by this approach. The resulting biosensor gave a linear concentration range of 0.5 to 93 mM and the shelf-life was up to 5 weeks at 4 °C.

Rebriiev and Starodub [102] developed a simple and rapid method for the immobilisation of urease on a ISFET gate surface based on the use of liquid photopolymerisable composite (LPhPC) as a target material. The use of a LPhPC enable the achievement of selective membranes for biosensors at a room temperature, with regulated physio-chemical properties of the resulting polymer and without application of a destructive temperature and chemical factors. N-vinyl pyrrolidone was used as a principal component of the LPhPC and was applied for immobilisation of urease [102]. The potentiometric biosensor gave a linear concentration range of 0.05 to 20 mM, a sensitivity of 38 mV decade^{-1} and was stable for about 40 days.

Eggenstein et al.[2] developed a potentiometric urea-sensitive biosensor based on a NH_4^+-sensitive disposable electrode in a double matrix membrane. The ion-sensitive polymer matrix membrane was formed in the presence of an additional electrochemical inert filter paper matrix to improve the reproducibility of the sensor production. A layer of urease was then cast onto the ion-sensitive membrane to enable the detection of urea. Poly(carbamoylsulfonate) hydrogel, produced from hydrophilic polyurethane pre-polymer blocked with bisulfite, served as the immobilisation material. The biosensor was successfully used for the direct determination of urea in blood samples. There was a good agreement between the results obtained with the biosensor and a spectrophotometric method. The biosensor achieved a detection limit of 20 µM and a linear concentration range of 72 µM to 21 mM. Fig. 6 shows the schematic of a disposable urea biosensor based on this construction design.

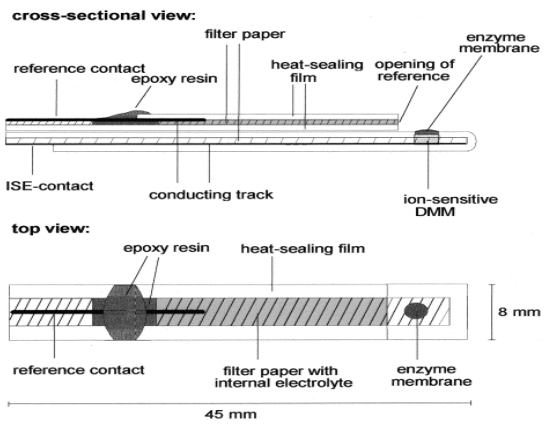

Fig. 6. Schematic diagram of a disposable urea potentiometric biosensor which consists of disposable measuring and reference electrode. Reproduced from [2].

Pan et al. [103] also used SiO_2 thin films on an ITO-FET to fabricate pH sensors. Photo-crosslinkable PVA bearing styrylpyridinium groups was then used to immobilise urease on the electrode. The resulting biosensor was stable for longer than 99 days and a linear concentration range of 0.8 to 13 mM was achieved for urea determination.

Boubriak et al. [32] constructed a urea biosensor by immobilisation of urease into to a BSA/glycerol membrane on the surface of an ISFET. However, the resulting potentiometric biosensor was not very sensitive achieving a sensitivity of 8 mV mM^{-1} with a linear concentration range from 0 to 2 mM. Nevertheless, the biosensor was successfully applied to the determination of urea in rat and rabbit blood serum samples.

Using a very different approach, Xu et al. [104] constructed a urea biosensor with a series piezoelectric crystal (SPC) device. This involves covalent immobilisation of urease layer on nylon nets or by fixing jack bean tissue slices on the probe surfaces of the SPC ammonia sensor. The urease sensor exhibits favourable frequency response to 0.01 to 3 mM urea with a response time of about 3 minutes.

3.3. Redox dyes and redox polymers

Another area that has attracted some interest for the fabrication of amperometric urea biosensors is the use of pH sensitive redox-active dyes [48-50]. Generally, these pH sensitive redox sensors rely on the influence of a pH change on formal redox potential of the redox dyes. Usually, the redox potential of the dye is shifted to more negative potentials with increase in pH and consequently this is accompanied by a detectable current change in an amperometric transducer when the working electrode is polarized at a constant potential. It follows therefore that when employed in urea biosensors, the addition of urea in the presence of urease results in a local pH-change on the electrode surface which is detected by the pH sensitive redox dyes. Pizzariello et al. [48] have used redox dyes, such as hematein [48], lauryl gallate [48] and methylene blue [49] for fabrication of amperometric urea biosensor based on this mechanism. The resulting biosensors exhibited linear concentration ranges from 0.025 to 0.35 mM, 0.002 to 0.75 mM, and 0.01 to 0.25 mM, respectively. Although the best linear concentration range and detection limit was obtained with lauryl gallate, it was found that the use of methylene blue gave the shortest response time (<30s). Also, like monomeric form of redox dyes, electrochemical properties of polyredox dyes is also strongly affected by the pH of the environment [50]. Furthermore, the electropolymerization of the redox dyes is a simple procedure for the preparation of stable layers on an electrode surface, as well as for immobilisation of urease into the growing polymeric network. Based on this principle, Vostiar et al. [50] prepared a polytoluidine blue (PTOB)-urease film by electropolymerisation and applied to the amperometric determination of urea, based on the above mechanism. A linear concentration range was achieved with the biosensor from 0.02 to 0.8 mM, with a detection limit of 0.02 mM and a response time of 20-30 s.

Koncki et al. [55-57] also prepared pH-sensitive membranes composed of Prussian blue (PB) doped with N-substituted polypyrroles for construction of an optical urea biosensor. The optical pH sensitivity of the film was associated with pH-dependent equilibria between fully isocyano hexacoordinated and partially hydrated high-spin Fe(III) ions in PB. These pH-dependent processes can be optically monitored at a wavelength of 720 nm, i.e., at the absorption maximum of PB. The organic polymer plays a double role in the composite material. It is a binding agent for inorganic compounds resulting in a high mechanical stability of the film. Moreover, the carboxylic groups of the organic component allow chemical binding of biomolecules to the film surface using a simple one-step carbodiimide method. Fig. 7 shows the FIA arrangement used with the optical biosensor for the determination of urea. The flow-through cell design that was adopted is fully compatible with conventional spectrophotometers. The optical biosensor achieved a detection limit of 0.1 mM and a linear concentration range of 0.2 to 100 mM [57]. They also applied the optical biosensor to the analysis of samples with a low buffering capacity, such as urine samples and saline extracts from pharmaceutical and cosmetic ointments containing urea [56] and also for on-line monitoring and control of hemodialysis therapy [55].

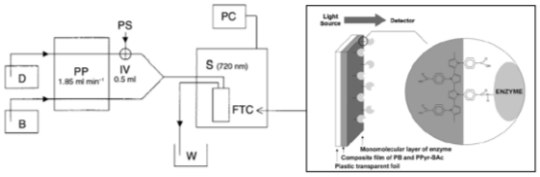

Fig. 7. Scheme of FIA manifold with a optical urea biosensor as detector: dialysate fluid (D), phosphate buffer (B), peristaltic pump (PP), injection valve (IV) for post-dialysate sample (PS), spectrophotometer (S) with flow-through cell (FTC; details in the inset) data storage/processing personal computer (PC) and waste (W) [55-57].

Fig. 8 shows the response obtained for urea in post-dialysate samples with the optical biosensor. In particular, the pH-enzyme optode-FIA system was useful for the selective determination of post-dialysate urea in the concentration range (2-16 mM) which corresponds to its level in clinical samples. This biosensor/FIA system enabled the analysis of about 15 samples of spent dialysate per hour and the operational and storage stabilities were longer than 2 weeks and 2 months, respectively.

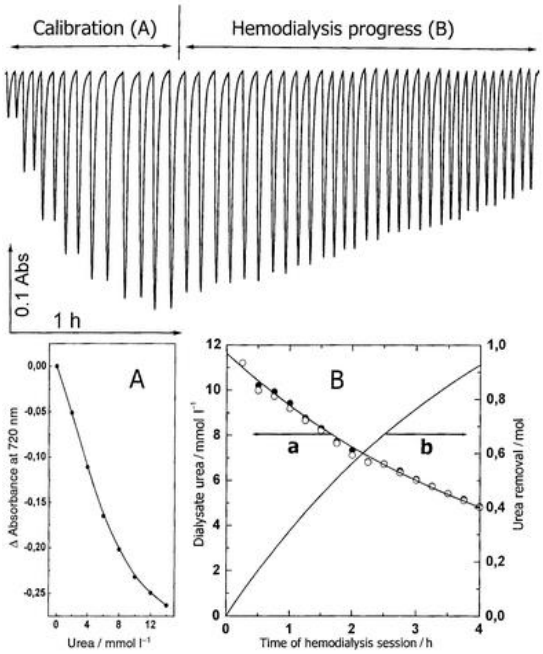

Fig. 8. Urea post-dialysate determinations made with a FIA-biosensor arrangement during a hemodialysis session. The standard dialysate fluid was used. (A) is for calibration with standards, and (B) is for post-dialysate sample measurements. Each urea standard and each sample of post-dialysate fluid were injected twice. Reproduced from [55].

Pizzariello et al. [48] also developed an amperometric urea biosensor by immobilising urease into a redox polymer matrix, poly(vinylferrocenium) (PVF^{+}), which has ion exchange properties. The enzyme urease (E) is negatively charged at a pH value above its isoelectric point and therefore, can be immobilised into the polymer matrix via anion exchange as illustrated by the following equation:

$$PVF^{+}ClO_4^{-} + E^{-} \rightarrow PVF^{+}E^{-} + ClO_4^{-} \quad (6)$$

During urea hydrolysis, urease immobilised in the PVF^{+} matrix produced a local pH change which significantly influences the formal redox potential of the $PVF^{+}ClO4^{-}$ redox polymer film. Consequently, at a suitable constant potential, the pH change of the system is accompanied by a detectable current change. The advantage of this biosensor sensor includes ease of preparation, long-term stability and reasonably fast response time (60 s). Also the biosensor achieved a detection limit of 1 µM and a linear concentration range of 1 to 250 µM. A potentiometric version of the biosensor was also reported, but with a higher detection limit of 5 µM and a wider linear range (0.05 to 100 mM) [105].

Milardovic et al. [106] also constructed a biosensor for urea based on the ion exchange ability of the mixed valence complex hexacyanoferrate anion, which is able to exchange univalent and divalent cations in crystal lattice/aqueous solutions. Depending on the iron oxidation state in the complex anion $(NiFe(CN)_6]_2$, the cations can enter or exit the crystal lattice in order to keep the surface layer electroneutral. This approach was used to develop an amperometric urea biosensor based on the electrocrystalization of nickel hexacyanoferrate and the subsequent immobilization of urease into this film. Consequently, the resulting ammonium ions from the biocatalytic reaction was exchanged on the transducer surface, as illustrated in equation 7, to produce a reduction current which is proportional to the ammonium ion concentration and, hence, to urea concentration:

$$\underset{\text{(film)}}{Na[NiFe^{III}(CN)_6} + \underset{\text{(electrod)}}{e^{-}} + \underset{\text{(solution)}}{NH_4^{+}} \rightarrow \underset{\text{(film)}}{NH_4Na[Ni\ Fe^{II}(CN)_6]} \quad (7)$$

3.4. Oxides, clays and zeolites

The use of inorganic materials such as oxides, clays and zeolites materials for enzyme immobilization and fabrication of biosensors has also been the subject of considerable research efforts [107]. In particular, clays are attractive materials for electrode functionalization of transducer surfaces because of their thermal stability, chemical inertness, well-defined layered structure, ion-exchange properties, and low cost. Swelling cationic clays, known as smectite clays, have been used for the fabrication of modified electrodes, particularly enzyme electrodes [108]. However, biomolecules often carry an overall negative charge, which limits their attraction by positively charged layers of these cationic clays. On the other hand, layered double hydroxides (LDH) can be synthesized as anionic clays, but apparently transport in this anionic LDH mirrors that of native cationic clays [108]. For this reason, these clays have an unusual anionic-charge property which can interact with anionic biomolecules [107,109]. Melo et al. [107] have used an anionic clay coating for the first time to develop sensitive urease based-FETs (ENFETs) for urea detection. A comparative study between

the properties of two different urea biosensors based on the immobilization of urease in two oppositely charged inorganic clay matrix (Laponite as a cationic clay and a LDH, $Zn_3Al(OH)_8Cl_2.H_2O$, as an anionic clay) demonstrated that ENFET based on LDH gave better properties, such as thermal stability (up to 45 °C versus 35 °C), storage stability (50 versus 15 days) and response time (5-10 s versus 1.5 min versus) than with laponite. While the main advantage of using laponite was its sensitivity, the good properties of LDH were due to electrostatic interactions between positively charged LDH and negatively charged urease. More recently, Nanohybrid of LDH and urease has been prepared for the first time by co-precipitation of enzyme and inorganic matrix [109]. The co-precipitation of LDH layers at constant pH between 7.0 and 8.0 prevents any deactivation of urease. This procedure decreased the immobilisation steps and preparation time than in an early work [107]. A linear concentration range of 0.1 to 12 mM was obtained for urea determination with this biosensor in the presence of tetraborate as urease inhibitor. Fig. 9 shows a model of the hybrid materials structure in which non-exfoliated and exfoliated layers co-existed in interactions with urease and, thus, compensating charge anions.

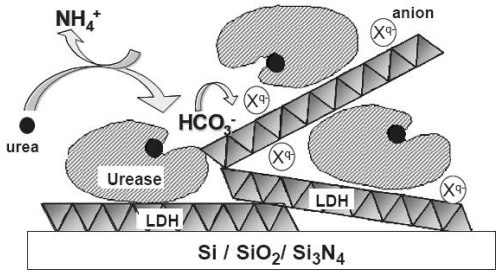

Fig. 9. Conceptual illustration of urease/LDH biomembrane used as part of an ENFET for urea determination [107, 109].

Vadgama and Crump [31] also prepared a potentiometric urea biosensor by covalent bonding of urease directly to the surface of an ammonium-FET. The NH_4^+-sensitive membrane is based on a zeolite-incorporated polymeric membrane (clinoptilolite). The grafting of urease onto the NH_4^+-sensitive membrane was performed by cross-linking with glutaraldehyde. The resulting urea ENFET achieved a relatively low sensitivity of 15 mV decade^{-1}, a detection limit of 30 µM and remained stable over 15 days.

Liu et al. [110] used the direct immobilisation of urease on Al_2O_3 substrate to develop a potentiometric biosensor for urea. The large specific surface area and porous structure of the $A1_2O_3$ particles resulted in high enzyme loading and achieved high sensitivity of 54 mV decade^{-1} for the biosensor. Also, the urease entrapped in this matrix was very stable due to the interactions of hydrogen bonding between Al_2O_3 and amine and carboxylic groups on the enzyme and a storage stability of up to 90 days was achieved.

More recently, Limbut et al. [111] immobilised urease on three different SiO_2-based matrices, including controlled pore glass (CPG), Poraver® and silica gel. The steps employed for the covalent bonding of urease to these silica based matrices are illustrated in Fig. 10.

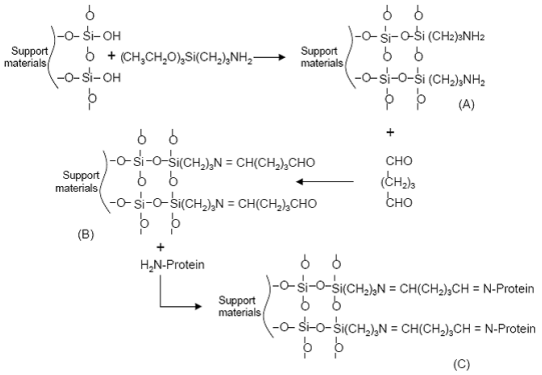

Fig. 10. Scheme for immobilisation of enzyme by covalent bonding on silica based matrices; (A) Silanization of the surface of support materials, (B) Primary amino acid groups ($R–NH_2$) of support are activated by glutaraldehyde ($C_5H_8O_2$) to give a carbonyl derivative by formation of a Schiff's base, (C) The formation of a Schiff's base linkage between carbonyl groups of the activated support and free amino groups on the protein [96].

These modified matrices were used in an enzyme column reactor coupled with a conductivity meter for conductometric detection of urea. The three enzyme reactors prepared with the different SiO_2-based matrices gave good responses for urea with linear concentration ranges between 5 and 45 mM. However, it was observed that the sensitivity of the enzyme reactor column prepared with Poraver® was 9.8 times less than for CPG and 9.2 times less than for silica gel. This may be due to the lower percentage of SiO_2 group in Poraver® (67%) which was 30% less than CPG (>97%) and 32% less than silica gel (>99%). The SiO_2 is important for the linkage between support and enzyme. Less SiO_2 means less immobilized enzyme and, thus, smaller response. Also, the surface area may be another factor. Both the particle size and pore size of Poraver® are much larger than those of CPG and silica gel. Therefore, for the same reactor volume the reactor prepared with Poraver® has much less surface area and hence less retention of the enzyme, Shu et al. [6] has also reported on the use of immobilised urease on CPG for the analysis of urea in blood. A

linear concentration range of 5 to 50 mM was achieved and the biosensor was operationally stable for 140 hours.

Tymecki et al. [112] has recently prepared a screen-printed metal-oxide based pH-electrode and found that urease/graphite paste doped with RuO_2 was still screen printable. Furthermore, thick-films fabricated using such biocomposite pastes were still pH-sensitive (with Nernstian slope of about 53 mV per pH), while still retaining the biocatalytic properties of the immobilised enzyme. Consequently, they developed system of planar electrodes which included the working and reference electrodes for use as a disposable device for drop-on measurements as well as for FIA-detection for multiple determination of blood urea nitrogen (BUN). The screen-printed biosensor achieved a linear concentration range of 1 to 31 mM and has a long operational lifetime (after 6 h of continuous measurements ≤10 % decay of initial sensitivity was observed). The device was successfully applied as BUN biosensor and detection element of hemodialysis monitor [113].

3.5. Sol-gel networks

Another interesting development which has impacted on the fabrication of urea biosensor is the use of sol–gel chemistry for construction of chemical sensors and biosensors. Sol–gel technology provides an attractive approach for the immobilisation of heat-sensitive bioactive substances, such as enzymes, proteins, and antibodies, due to the inherently low temperature of the associated process. The class of sol–gel silicate matrix used in this process permits chemical inertness, physical rigidity, negligible swelling in aqueous solution, tunable porosity, high photochemical and thermal stability, and optical transparency [106,114]. These attractive features have led to an intense research in this area mainly based on optical and electrochemical detection methods.

Lee et al. [37] developed conductometric urea biosensors based on sol-gel immobilisation of urease with the use of tetramethoxysilane (TMOS) as a sol-gel precursor. They also constructed a conductometric biosensor with a sol-gel immobilised urease on two different substrates: (a) on a gold interdigitated array (IDA) electrode constructed using standard photolithographic technology [36], and (b) on a platinum IDA electrode constructed by screen printed technology [37]. The use of the thick-film IDA electrodes were found to be excellent conductometric transducers in which the admittance signals were dominated by the conductance signals. The linear concentration ranges achieved with the two different substrates were 0.2 to 50 mM and 0.03 to 30 mM, respectively, and the electrodes enabled the determination of urea in serum and urine.

Sheppard et al. [115] also prepared SiO_2 network on a platinum IDA electrode with 3-aminopropyltrimethoxysilane (APTMS) as precursor. Free amine functional groups on the sol-gel network were employed for covalent attachment of urease. The resulting biosensor enabled conductometric determination of urea within a concentration range of 0.01 to 5 mM.

Agnieszka et al. [116] studied the influence of urease as an organic dopant on sol-gel optical properties in visible range and constructed a urea optode by entrapment of bromothymol blue as an optical pH indicator with urease in the sol-gel network [59]. The change of pH in the presence of urea causes the color change in bromothymol blue that can be detected spectrophotometrically. This optode was useful for the determination of urea within 3.5 to 9 mM.

Tsai and Doong. [58] used TMOS as a sol-gel precursor for entrapment of enzymes and their immobilisation on a glass micro array. The resulting array-based biosensor enabled simultaneous determination of urea and acetylcholine based on the detection of the increased pH caused by urea-urease system or of the pH reduction caused by acetylcholine-acetylcholinesterase and their effect on the fluorescent intensity of FITC-dextran as a fluorescent agent. A detection limit of 2.5 µM was achieved and the linear concentration range was 0.1 to 10 mM. Also heavy metals were detected with this array biosensor based on the inhibitory effects on urease.

Kondoh et al. [65] also immobilised urease on a surface acoustic wave device via a sol-gel layer. Sol-gel layer synthesized by using APTMS which contains amine functional groups can combine with glutaraldehyde to form a Schiff base for attachment to urease. A linear concentration range of 0.07 to 5 mM of urea was achieved with the biosensor.

3.6. Nanomaterials

In recent years, nanomaterials such as metal nanoparticles, carbon nanotubes, conductive nano polymers and other nanomaterials have applied as new matrixes for immobilization of urease in the various surfaces/transducers [81, 147-152, 154-161]. Significant advances have been made in synthetic methodologies such that it is now possible to prepare a variety of nanomaterials with highly controllable size, shape, surface charge and physicochemical characteristics. Two major advantages of nanomaterials are their

potential to be utilized as non-invasive diagnostic tools and the capacity for combining multiple modalities within a single probe. This enables far higher sensitivities to be achieved, which leads to further clarity and deeper insights into in vivo processes. Also, given their sensitivity, flexibility and miniaturization, these sensors may serve as a new paradigm for clinical and field-deployable analytical instruments.

Sevik et al. [160] reported a novel urea biosensor based on immobilizing urease on the surface of Fe3O4 nanoparticle modified Au electrode. The developed biosensor shows a rapid response, a wide linear range, and a high sensitivity of urea detection.

Srivastava et al. [159] demonstrated the fabrication of a highly sensitive mediator-free microfluidics sensor comprising of PDMS microchannels, patterned electrodes for the rapid detection of urea. urease and GLDH have been successfully co-immobilized onto TiO_2–ZrO_2 nanocomposite microelectrodes surface. This mediator-free microfluidics sensor offers improved sensitivity, detection limit and fast response time. This is attributed to the good electrocatalytic behavior of the nanocomposite as well as smaller geometry of the sensor.

A novel amperometric biosensor for the detection of urea was prepared by immobilizing urease on a modified electrode of nanocomposite film/platinum substrate [157]. The nanocomposite film was obtained by electrochemical co-polymerization of the corresponding monomers in the presence of functionalized carbon nanotubes and different additives. The immobilized urease on the nanocomposite film surface exhibited an excellent electrocatalytic response toward the reduction of urea.

Nouira et al. [156] presented that the use of urease functionalized gold nanoparticles allows the amplification of the conductivity variation during enzymatic urea hydrolysis and to enlarge the domain of detected concentrations. This phenomenon can be due to the decrease of the probed thickness with the addition of gold nanoparticles.

A novel potentiometric urea biosensor has been fabricated with urease immobilized multi-walled carbon nanotubes embedded in silica matrix deposited on the surface of indium tin oxide [150] coated glass plate. The synergistic effect of silica matrix, MWCNTs and their biocompatibility with urease made the biosensor to have the excellent electro catalytic activity and high stability. The resulting biosensor exhibits a good response performance to urea detection with a wide linear range.

3.7. Other matrices and approaches

Various other matrices have used for construction of urea biosensors, such as carbon paste [4], fullerene C60, anion exchange resin, soybean tissue, agarose bead and epoxy activated support. Seo et al. [24] developed an enzyme-modified carbon paste electrode by incorporating urease and GDH into carbon paste for amperometric urea determination. Urea determination is based on oxidation current of NADH monitored at 1.1 volt vs. Ag/AgCl. A detection limit of 0.005 mM and a linear concentration range of 0.02 to 0.2 mM was achieved with the biosensor. Also in the same approach, they [25] immobilized these two enzymes onto a commercial membrane (immobilon-AV affinity membrane) and placed it on the glassy carbon electrode. This biosensor enabled amperometric determination of urea within a linear concentration range of 0.02 to 0.2 mM.

Cunningham [117] attached urease covalently on a commercial membrane and then used ultrasonic welding to reliably attach immobilised urease membrane over an ammonium ion selective electrode for construction of a potentiometric biosensor for detection of urea in peritoneal dialysis fluids. The sensitivity of the biosensor to urea in the stopped-flow system was very low at 2.5 mV sec^{-1} per decade in the concentration range from 0.5 mM to 15 mM urea.

Wei and Shih [66] developed a piezoelectric biosensor for urea by coating an immobilised C60/urease enzyme/PVC membrane on piezoelectric crystals [66]. Fullerene C60 was used as the coating material to adsorb and detect NH_4^+ generated by the catalytic reaction. The resulting piezoelectric urea sensor exhibited a linear concentration range from 0.1 to 100 mM and demonstrated a good selectivity in presence of some common compounds in biological systems.

Pijanowska et al. [118] used glutaraldehyde and the Schiff base formation to directly attach urease on transducer surface as a basis for fabrication of a urea biosensor based on an enzyme-modified pH-sensitive Si_3N_4 gate ISFET. The biosensor exhibited a linear concentration range from 0.3 to 10 mM.

Qin et al. [119] developed a novel plant tissue-based chemiluminescence (CL) biosensor for the detection of urea. The analytical reagents

involved in the CL reaction, including luminol and permanganate, were both immobilised on anion exchange resin columns, while soybean tissue was packed in a mini-glass column. The urease in the plant tissue column catalysed the hydrolysis of urea to NH_4^+ and HCO_3^-. The anion produced released luminol from the luminol-immobilised anion-exchange column, which then reacted with permanganate eluted with sodium hydroxide from the permaganate immobilised anion-exchange column, thus producing a CL signal. The CL biosensor achieved a detection limit was 2 μM and a linear concentration range of 4–400 μM;.

Bjarnason et al. [120] immobilised urease onto a commercial epoxy activated support (Poros 20 EP). The resulting biomaterial was used in an enzyme reactor and the heat produced from the hydrolysis of urea by urease was measured with a thermocouple located close to the enzyme reactor. The thermal biosensor achieved a linear concentration range of 0.5 to15 mM, with a detection limit of 0.5 mM. Up to 60 samples per hour were analysed with the thermal biosensor.

Xie et al. [121-122] also developed an integrated thermal biosensor arrays for the simultaneous determination of glucose, penicillin and urea. The enzymes were immobilized on NHS-activated agarose beads which were then sequentially packed into distinct regions of the microchannel. Each of the regions contained one immobilized enzyme matrix and a pair of thermistors for differential measurement of temperature changes in association with the enzyme reaction. The thermal biosensor arrays achieved linear concentration ranges of 1 to 25 mM [121] and 0.5 to 20 mM [122] for urea.

4. CHOICE TRUNSDUCERS FOR UREA BIOSENSORS

In addition to the choice of a suitable enzyme or other bioactive substance and immobilisation method, a critical consideration for achieving adequate detection of urea with a biosensor is the appropriate choice of a suitable mode of transduction of the analytical signal. The transducer is needed to convert the biochemical signal associated with the enzyme-catalyzed reaction to an electronic signal. Depending on the nature of the biochemical reaction involved, one or more mode of transduction may be applicable to a sensor assembly [26-29]. The range of transducers that have been used for fabrication of urea biosensors include potentiometric, amperometric, conductometric, optical, thermal, manometric and piezoelectric transducers (see Table 2, third column). Some of these modes of transduction were referred to in the discussion in section 3, but the merits and problems associated with each of these modes of transduction were not discussed and are thus covered briefly below.

4.1. Potentiometric Detection

One of the most attractive modes of detection of urea commonly used in many of the reported urea biosensors is based on the potentiometric detection of ammonium ion, ammonia gas, carbon dioxide or pH changed generated by the enzymatic hydrolysis of urea. Notably, the requireded devices for this mode of transduction are simple to construct and are readily available [30].

As the biocatalytic reaction of urease primarily results in a pH change, most urea biosensors are based on potentiometric mode of pH detection. However, a major problem for pH-sensitive electrodes is that the sensor response is strongly dependent on the buffer capacity of the sample solution. Therefore the small pH change produced by the enzyme-catalysed reaction may be suppressed when a high buffer concentration is employed and can lead to a narrow dynamic range and in some case loss in sensor sensitivity [3,31].

The other common potentiometric transducer which is used to detect ammonium ions generated from the enzymatic hydrolysis of urea and also based on monitoring potential change is an ion-selective electrode. However, the performance of this NH_4^+-sensitive electrode can be affected by interference from Na^{+} and K^{+} ions, which are commonly present in samples, such as serum (4.5 mM K and 140 mM Na) and urine [3,32]. Nevertheless, it is possible to incorporate an additional membrane barrier to improve the performance of some ion-selective electrodes. For example, it has been found that the coverage of an NH_4^+ electrode with ion-exchange membrane which contained quaternary ammonium ions is useful for rejecting interfering K^+ ions in solution and for avoiding errors associated with changes in background NH_4^+ ions [33].

For gaseous substances, such as CO_2 and NH_3, gas potentiometric sensors are often used, but their response is largely independent of sample matrix effects. This means that any background variation in the detected gaseous species requires compensation. Thus, this approach often requires the addition of a gas-selective membrane to provide a further diffusion barrier that reduces sensitivity and extends response time [33].

4.2. Conductometric Detection

Transduction based on conductometric measurement is also useful for biosensing of urea, based on its ability to detect the change in solution resistance (reciprocal of conductance)

during enzymatic reaction. These sensors are also simple to construct, suitable for miniaturization and mass production. In addition, conductometric sensors do not require the use of a reference electrode and are unaffected by colour or turbidity [34], but can suffer from lack of specificity. Substances that can contribute to the conductivity of a solution can influence the reliability of its use.

In an early research on conductometric enzyme assay, Lawrence and Moores [35] described five categories of enzymes that, separately or in combination, allow the application of conductometric methods to enzymatic reactions. These enzymes were: (a) amidases for generation of ionic groups, (b) dehydrogenases and decarboxylases for resulting in separation of unlike charges, (c) estrases for protein migration, (d) kinases for changing the degrees of association of ions, and (e) phosphatases and sulfatases for causing in change in size of charge–carrying groups. Among these, the urease-catalysis hydrolysis of urea is ideally suited to conductometric mode of detection. The three ions (NH_4^+, HCO_3^- and OH^-) produced from a single uncharged urea molecule can contribute to a significant increase in ionic strength. It has been reported that good S/N can be achieved if the initial ionic strength (buffer concentration) of the assay medium is low [34]. Lee et al. [36,37] have used sol-gel immobilised urease on planar interdigitated electrode arrays for fabrication of urea conductometric biosensors [36-37]. In this case, the charged products of the urease-catalyzed hydrolysis of urea increase the solution conductivity around the vicinity of the sensor surface and the measured conductivity is related to urea concentration.

4.3. Amperometric Detection

The most widely used mode of transduction with most biosensors is by amperometric detection. This mode of detection involves the measurement of resulting cathodic or anodic current following the application of a fixed potential to the biosensor. The detection can be based on either the oxidation or reduction of products or reactants of the enzymatic reaction [39]. Despite its better sensitivity and lower detection limit compared with those obtained by potentiometric methods [23,40], it can suffer from some limitations. In some cases, the conventional immobilisation of urease in or onto non-conducting membranes may make it difficult to observe the current change which results from the biocatalytic reaction [41]. In addition, the products of the enzymatic hydrolysis of urea are not good electroactive compounds. So often for urea amperometric biosensor, the use of conducting polymers [4,23,41-46], redox polymers [47] and redox dyes [48-50] are considered as a means of overcoming this limitation (Table 2, second and third column). In these cases, the products of urease-catalysed hydrolysis of urea cause changes in the redox properties of these matrices and, hence, result in a change in the current response. On the other hand, the inclusion of an additional enzyme or co-factor, such as glutamate dehydrogenase or NAD(H) are employed to generate amperometric responses from electroinactive product(s) [21,24-25,51].

4.4. Optical Detection

The use of optical chemical sensors has also attracted some interests as an alternative means of detection of urea. Several optical biosensors have been described for urea determination that involve immobilisation of urease onto different matrices, such as PVC [52-54], N-substituted polypyrrole [55-57] or sol-gel [58-59], together with a pH indicator or an ammonia-specific indicator. A distinct disadvantage of using optical biosensors for urea is that none of the catalytic products (ammonia or carbon dioxide) or the analyte show optical properties for adequate detection by optical sensors. For this reason, a second reaction or an indicator dye is often used to achieve adequate detection. An exceptional example of an optical urea biosensor which does not require such reaction or the addition of dye has been reported by de Marcos et al. [60] and it is based on the use of a conductive polymer (polypyrrole) and has been successfully used for urea determination in biological samples. The main advantage of this conducting polymer-based optical biosensor is that the matrix itself acts both as a support and as an optical indicator. The absorbance spectrum of conductive polymer depends on pH and it changes in the presence of the catalytic product generated from the urease-catalysed hydrolysis of urea [60].

4.5. Manometric Detection

The measurement of the total quantity of a gas (such as NH_3 or CO_2) produced during the enzymatic hydrolysis of urea have also been made by simple manometric methods [61-62]. These methods are based on the ideal gas law, which states that the volume occupied by a gas is directly proportional to the number of moles of the gas at constant pressure and temperature. The gas volume is thus measured as a function of time or at completion of the reaction [61,63]. Using this principle, Jenkins and Delwiche [11] developed an on-line biosensor and use this to measure urea in milk with a piezoresistive

pressure transducer during milking. The sensor is robust to milk and the complete measurement cycle, including a wash cycle between samples and correction for background dissolved gases, lasted about 5 min [11]. The main disadvantage of these manometric sensors compared to other modes of transduction is that the devices could only be used for the analysis of discrete samples in a sealed system. Another limitation arose from practical considerations of the dimension of the headspace gas volume relative to the sample volume. For effective mass transfer and reproducible sensitivity, this volume was required to be relatively large [62]. This can result in a loss of sensitivity compared to the theoretical maximum. Jenkins and Delwiche [62] proposed a modification to solve these problems wherein the gaseous volume and pressure sensor would be contained within a porous membrane. Soluble gases could then move across the membrane to and from the sample, but the gas phase would be held inside the cavity due to the surface tension of the sample on the membrane. The cavity pressure could then be independent of the pressure in the sample, and the entire sensor could be immersed in the sample. Furthermore, the volume of the gaseous cavity could be made constant and much smaller so that the sensitivity of the sensor is reproducible and as large as possible [62].

4.6. Thermal Detection

The detection of thermal change has not gained much interest for urea biosensors, but has been used in some cases. These are based on the measurement of the heat produced when a biochemical reaction takes place. The amount of reacted substrate is related to the heat produced through the specific enthalpy, ΔH, of the reaction. An advantage of this approach, compared to other analytical modes of detection such as spectrophotometric and electrochemical methods, is the universal detection principle on which it is based combined with the specificity of biological reactions. As all biological reactions are exothermic, this principle is applicable as long as suitable specificity is obtained in the enzymatic reaction [61]. However, a major drawback of this device is that non-specific heat effects from mixing, changes in pH, viscosity and ionic strength can also produce signals. The sensitivity of this method is directly related to the apparent molar enthalpy of a reaction and, as such, only more endo- or exothermic reactions are often considered for detection by this approach [39]. Jerspersen [64] has reported that urease–catalysed urea hydrolysis reaction has an enthalpy of -14.97 Kcal mol^{-1} [64] which is adequate for thermal biosensing of urea.

4.7. Piezoelectric Detection

Another transduction mode that has gained some interest for biosensing of urea is piezoelectric detection. In general, the detection of the change in mechanical characteristics such as mass loading effect and elastic characteristic due to immunity reaction on a propagation plane or electrode can be achieved with an acoustic device. However, the acoustic sensor which normally detects change in mechanical properties cannot detect an enzymatic reaction. By using a piezoelectric crystal in the acoustic device, a piezoelectric field is formed according to the wave propagation. Since the piezoelectric field penetrates into the medium in contact with the device, the electrical properties (dielectric contact and conductivity) of the medium can be detected. Kondoh et al. [65] used this approach to develop urea piezoelectric biosensors, where the interaction between immobilised urease on these devices and urea in solution is detected based on change in conductivity monitored by acoustoelectric interaction. Wei and Shih [66] used another approach which involved the coating of piezoelectric surface with an adsorbent which can adsorb the products of the enzymatic reaction (ammonia or carbon dioxide) and, thus, serve as a basis for measurement of urea concentration [66].

5. CONCLUSION

As with other biosensors, successful fabrication of urea biosensors requires careful consideration of several important factors, including choice of enzyme or other bioactive substances, immobilisation method and mode of transduction. However, if done properly, this can lead to successful determination of urea in various clinical, environmental and industrial samples, as has been demonstrated in this review. Without doubt, the various urea biosensors that have been reported to date have had many positive impacts in the analysis of urea in various samples such as urine, blood, milk, wine, coastal waters, fertilizers, soil, cosmetic and pharmaceutical samples. Recent developments in this area is encourage a more common use of nanomaterials for the fabrication of more robust and more sensitive urea biosensors.

REFERENCES

[1] P.S. Francis, S.W. Lewis and K.F. Lim, Analytical methodology for the determination of urea: current practice and future trends, Trends in analytical chemistry, *Trends Anal. Chem.* 21 (2002) 389-400.

[2] C. Eggenstein, M. Borchardt, C. Diekmann, B. Gründig, C. Dumschat, K. Cammann, M. Knoll and F. Spener, A disposable biosensor

for urea determination in blood based on an ammonium-sensitive transducer, *Biosens. Bioelectron.* 14 (1999) 33–41.

[3] E. Karakus, S. Pekyard and E. Klc, urea biosensors based on PVC membrane containing palmitic acid, *Artif. Cell. Blood Sub*. 33 (2005) 329–341.

[4] Y.C. Luo and J.S. Do, Urea biosensor based on PANi(urease)-Nafion/Au composite electrode, *Biosens. Bioelectron.* 20 (2004) 15–23.

[5] Wałcerz, S. Głab and R. Koncki, Potentiometric enzyme electrode in a flow injection system for the determination of urea in human serum samples, *Anal. Chim. Acta* 369 (1998) 129–137.

[6] H.C. Shu and N. Wu, Continuous analysis of urea in blood by immobilized urease using conductive biosensor, *J. Chin. Chem. Soc*. 47 (2000) 987-991.

[7] T.A. Yamarik and A.R. Elmore, Final report of the safety assessment of urea, *Int. J. Toxicol*. 24 (2005) 1–56.

[8] A.J. Taylor and P. Vadgama, Analytical reviews in clinical biochemistry: the estimation of urea, *Ann. Clin. Biochem.* 29 (1992) 245-264.

[9] J.S. Jonker, R.A. Kohn and R.A. Erdman, Using milk urea nitrogen to predict nitrogen excretion and utilization in lactating dairy cows, *J. Dairy Sci.* 81 (1998) 2681-2692.

[10] R. Kohn, Caution needed when interpreting MUNs, *Hoard's Dairyman* 145 (2000) 58-69

[11] D.M. Jenkins and M.J. Delwiche, Manometric biosensor for on-line measurement of milk urea, *Biosens. Bioelectron*. 17 (2002) 557-563.

[12] C.E. Butzke and L.F. Bisson, Ethyl Carbamate Preventative Action Manual, US Food and Drug Administration, Center for Food Safety and Applied Nutrition, 1997.

[13] P.M. Glibert, J. Harridon, C. Heil and S. Seitzinger, Escalating worldwide use of urea–a global change contributing to coastal eutrophication, *Biogeochem.* 77 (2006) 441–463.

[14] P.M. Glibert, T.M. Trice and B. Michael, Lane, Urea in the tributaries of the Chesapeake and coastal bays of Maryland, *Water Air Soil Pollut.* 160 (2005) 229–243.

[15] P.M. Glibert, S. Seitzinger, C.A. Heil, J.A. Burkholder, M.W. Parrow, L.A. Codispoti and V. Kelly, The role of eutrophication in the global proliferation of harmful algal blooms: new perspectives and approaches, *Oceanogr*. 18 (2005) 196–207.

[16] M. Revilla, J. Alexander and P.M. Glibert, Urea analysis in coastal waters: comparison of enzymatic and direct methods, *Limnol. Oceanogr*. 3 (2005) 290–299.

[17] D.E. Lamber, J.E. Sherwood and P.S. Francis, The determination of urea in soil extracts and related samples-a review, *Aust. J. Soil Res.* 42 (2004) 709-717.

[18] A.R. Butler and D. Walsh, Colorimetric non-enzymic methods for the determination of urea, *Trends Anal. Chem.* 1 (1982) 120-124.

[19] E. Jabri, M.B. Carr, P.A. Karplus and R. Hausinger, The crystal structure of urease from klebsiella aerogenes, *Science* 268 (1995) 998-1004.

[20] G. Mamiya, K. Takishima, M. Masakuni, T. Kayumi and K. Ogawa, Complete amino acid sequence of jack bean urease, *J. Protein Chem.* 6 (1987) 55-59.

[21] A. Gambhir, M. Gerard, A. K. Mulchandani, B. D. Malhotra, Coimmobilization of urease and glutamate dehydrogenase in electrochemically prepared polypyrrole-polyvinyl sulfonate films, *Appl. Biochem. Biotechnol.* 96 (2001) 249-257.

[22] G.G. Guilbault, R.K. Smith and J.G. Montalvo, Use of ion selective electrodes in enzymic analysis. Cation electrodes for deaminase enzyme systems, *Anal. Chem.* 41 (1969) 600-605.

[23] W.J. Cho and H.J. Huang, An amperometric urea biosensor based on a polyaniline-perfluorosulfonated ionomer composite electrode, *Anal. Chem.* 70 (1998) 3946-3951.

[24] J.K. Yang, K.S. Ha, H.S. Baek, S. S. Lee and M.L. Seo, Amperometric determination of urea using enzyme-modified carbon paste electrode, *Bull. Korean Chem. Soc.* 25 (2004) 1499-1502.

[25] K.S. Ha and M.L. Seo, Amperometric biosensor for urea, *Bull. Korean Chem. Soc.* 18 (1997) 1149-1152.

[26] D.D. Borole, U.R. Kapadi, P.P. Mahulikar and D.G. Hundivale, Conducting polymers: an emerging field of biosensors, *Des. Monomers Polym.* 9 (2006) 1–11.

[27] A. Ramanavicius, A. Ramanaviciene and A. Malinauskas, Electrochemical sensors based on conducting polymer-polypyrrole, *Electrochim. Acta* 51 (2006) 6025-6037.

[28] D. Malhotra, A. Chaubey and S.P. Singh, Prospects of conducting polymers in biosensors, *Anal. Chim. Acta* 578 (2006) 59-74.

[29] M. Gerard, A. Chaubey and B.D. Malhotra, Application of conducting polymers to biosensors, *Biosens. Bioelectron.* 17 (2002) 345–359.

[30] M.L. Hamlaoui , K. Reybier, M. Marrakchi, N. Jaffrezic-Renault, C. Martelet, R. Kherrat

and A. Walcarius , Development of a urea biosensor based on a polymeric membrane including zeolite, *Anal. Chim. Acta* 466 (2002) 39–45.

[31] P. Vadgama and P.W. Crump, Biosensors: recent trends, *Analyst* 117 (1992) 1657-1670.

[32] O.A. Boubriak, A.P. Soldatkin, N.F. Starodub, A.K. Sandrovsky and A.K. Elskaya, Determination of urea in blood serum by a urease biosensor based on an ion-sensitive field-effect transistor, *Sens. Actuators B* 26-27 (1995) 429-431.

[33] S.A. Rosario, G.S. Cha, M.E. Meyerhoff and M. Trojanowicz, Use of ionomer membranes to enhance the selectivity of electrode-based biosensors in flow-injection analysis, *Anal. Chem.* 62 (1990) 2418-2424.

[34] H. Ghourchian, A.M. Rad and H. Elyasvandi, A Conductometric urea biosensor by direct immobilization of urease on Pt electrode, *Irainian J. Chem. Chem. Eng.* 23 (2004) 55-63.

[35] J. Lawrence and G.R. Moores, Conductometry in enzyme studies, *Eur. J. Biochem.* 24 (1672) 538-546.

[36] W.Y. Lee, K.S. Lee, T.H. Kim and J.K. Part, Microfabricated conductometric urea biosensor based on sol-gel immobilized urease, *Electroanalysis* 12 (2000) 78-82.

[37] W.Y. Lee, S.R. Kim, T.H. Kim, K.S. Lee, M.C. Shin and J.K. Park, Sol–gel-derived thick-film conductometric biosensor for urea determination in serum, *Anal. Chim. Acta* 404 (2000) 195–203.

[38] M. Trojanowicz and T.K.V. Krowczyk, Electrochemical biosensors based on enzymes immobilized in electropolymerized films, *Mikrochim. Acta* 121 (1995) 167-181.

[39] A.O. Scott, *Biosensors for food analysis, The Royal Society of Chemistry*, UK, 1998, PP: 13-28.

[40] P.C. Pandey and A.P. Mishra, Conducting polymer-coated enzyme microsensor for urea, *Analyst* 113 (1988) 329-331.

[41] S. Adeloju, S.J. Shaw and G.G. Wallace, Polypyrrole-based amperometric flow injection biosensor for urea, *Anal Chim. Acta* 323 (1996) 107-113.

[42] B. Strehlitz, B. Gründig and H. Kopinke, Sensor for amperometric determination of ammonia and ammonia-forming enzyme reactions, *Anal. Chim. Acta* 403 (2000) 11-23.

[43] S.B. Adeloju, S.J. Shaw and G.G. Wallace, Pulsed-amperometric detection of urea in blood samples on a conducting polypyrrole-urease biosensor, *Anal. Chim. Acta* 341 (1997) 155-160.

[44] M. Trojanowicz, A. Lewenstam, T.K. Vel Krawczyk, I. Lahdesmaki and W. Szczepek, Flow injection amperometric detection of ammonia using a polypyrrole-modified electrode and its application in urea and creatinine biosensors, *Electroanalysis* 8 (1996) 233-243.

[45] V. Bisht Rajesh, W. Takashima and K. Kaneto, An amperometric urea biosensor based on covalent immobilization of urease onto an electrochemicallyprepared copolymer poly (N-3-aminopropyl pyrrole-co-pyrrole) film, *Biomaterials* 26 (2005) 3683–3690.

[46] J.H. Jin, S.H. Paek, C.W. Lee, N.K. Min and S. I. Hong, Fabrication of amperometric urea sensor based on nano-porous silicon technology, *J. Korean Phys. Soc.* 42 (2003) 735-738.

[47] F. Kuralay, H. Ozyoruk and A. Yıldız, Potentiometric enzyme electrode for urea determination using immobilized urease in poly(vinylferrocenium) film, *Sens. Actuators B* 109 (2005) 194–199.

[48] A. Pizzariello, M. Stredansky and S. Stredanska, S. Miertus, Urea biosensor based on amperometric pH-sensing with hematein as a pH-sensitive redox mediator, *Talanta* 54 (2001) 763–772.

[49] M. Stredansky, A. Pizzariello, S. Stredanska and S. Miertus, Amperometric pH-sensing biosensors for urea, penicillin, and oxalacetate, *Anal. Chim. Acta.* 415 (2000) 151–157.

[50] I. Vostiar, J. Tkac, E. Sturdik and P. Gemeiner, Amperometric urea biosensor based on urease and electropolymerized toluidine blue dye as a pH-sensitive redox probe, *Bioelectrochem*. 56 (2002) 113– 115.

[51] P. Bertocchi, D. Compagnone and G. Palleschi, Amperometric ammonium ion and urea determination with enzyme-based probes, *Biosens. Bioelectron.* 11 (1996) 1-10.

[52] B. Kovacs, G. Nagy, R. Dombi and K. Toth, Optical biosensor for urea with improved response time, *Biosens. Bioelectron.* 18 (2003) 111-118.

[53] R. Koncki, G.J. Mohr and O.S. Wolfbeis, Enzyme biosensor for urea based on a novel pH bulk optode membrane, *Biosens. Bioelectron.* 10 (1995) 653-659.

[54] H. Chen and E. Wang, Optical urea biosensor based on ammonium ion selective membrane, *Anal. Lett.* 33 (2000) 997-1011.

[55] A. Radomska, S. Glab and R. Koncki, Spectrophotometric bioanalytical flow-injection system for control of hemodialysis treatment, *Analyst* 126 (2001) 1564–1567.

[56] R. Koncki, T. Lenarczuk, A. Radomska and

S. Glab, Optical biosensors based on Prussian Blue films, *Analyst* 126 (2001) 1080–1085.

[57] R. Koncki and O.S. Wolfbeis, Composite films of Prussian blue and *N*-substituted polypyrroles: covalent immobilization of enzymes and application to near infrared optical biosensing, *Biosens. Bioelectron.* 14 (1999) 87–92.

[58] H.C. Tsai and R. Doong, Simultaneous determination of pH, urea, acetylcholine and heavy metals using array-based enzymatic optical biosensor, *Biosens. Bioelectron.* 20 (2005) 1796-1804.

[59] A. Olatowska-Jarza and H. Podbielska, Biosensor for urea detection based on sol-gel technology, *Optica Applicata* 32 (2002) 685-690.

[60] S. de Marcos, R. Hortiguela, J. Galban, J.R. Castillo and O.S. Wolfbeis, Characterization of a urea optical sensor based on polypyrrole, *Mikrochim. Acta* 130 (1999) 267-272.

[61] S.R. Mikkelsen and E. Corton, *Bioanalytical Chemistry*, John Wiley & Son, New Jersey, 2004, Chapter 3.

[62] D.M. Jenkins and M.J. Delwiche, An immersible manometric sensor for measurement of humidity and enzyme mediated changes in dissolved gas, *Biosens. Bioelectron*. 18 (2003) 1085-1093.

[63] M. Jenkins, M.J. Delwiche, E.J. DePeters and R.H. BonDurant, Chemical assay of urea for automated sensing in milk, *J. Dairy Sci.* 82 (1999) 1999-2004.

[64] N.D. Jespersen, **A** Thermochemical Study of the Hydrolysis of Urea by Urease, *J. Am. Chem. Soc*. 97 (1975) 1662-1667.

[65] J. Kondoh, T. Imayama, Y. Matsui and S. Shiokawa, Enzyme biosensor based on surface acoustic wave device, *Electron. Commun. Jpn*. 79 (1996) 69-75.

[66] L.F. Wei and J.S. Shih, Fullerene-cryptand coated piezoelectric crystal urea sensor based on urease, *Anal. Chim. Acta* 437 (2001) 77-85.

[67] I. Walcerz, R. Koncki, E. Leszczynska, B. Salamonowicz and S. Glab, Urea biosensors based on PVC membrane ion-selective electrodes, *Anal. Lett.* 29 (1996) 1939-1953.

[68] T. Hianik, Z. Cervenanska, T. Krawczynski vel Krawczyk and M. Snejdarkova, Conductance and electrostriction of bilayer lipid membranes supported on conducting polymer and their application for determination of ammonia and urea, *Mater. Sci. Eng. C* 5 (1998) 301-305.

[69] S. Komaba, M. Seyama, T. Momma and T. Osaka, Potentiometric biosensor for urea based on electropolymerized electroinactive polypyrrole, *Electrochim. Acta* 42 (1997) 383-388.

[70] C. Hernandez, A. Witkowski, S. Daunert and L.G. Bachas, Potentiometric enzyme electrode for urea based on electrochemically prepared polypyrrole membranes, *Mikrochim. Acta* 121 (1995) 63-72.

[71] T. Osaka, S. Komaba, M. Seyama and K. Tanabe, High-sensitivity urea sensor based on the composite film of electroinactive polypyrrole with polyion complex, *Sens. Actuators B* 35-36 (1996) 463-469.

[72] T. Osaka, S. Komaba, Y. Fujino, T. Matsuda and I. Satohb, High sensitivity flow injection analysis of urea using composite electropolymerized polypyrrole-polyion complex film, *J. Electrochem. Soc.* 146 (1999) 615-619.

[73] A. Gambhir, A. Kumar, B.D. Malhotra, B. Miksa and S. Slomkowski, Covalent immobilization of urease on polypyrrole microspheres for application as a urea biosensor, *E-Polymers* 52 (2002) 1-9.

[74] V. Bisht Rajesh, W. Takashima and K. Kaneto, A novel thin film urea biosensor based on copolymer poly(N-3-aminopropy-lpyrrole-co-pyrrole) film, *Surf. Coat. Technol*. 198 (2005) 231– 236.

[75] V. Bisht Rajesh, W. Takashima and K. Kaneto, Development of a potentiometric urea biosensor based on copolymer poly(N-3-aminopropyl pyrrole-co-pyrrole) film, *React. Funct. Polym.* 62 (2005) 51–59.

[76] A. Senillou, N. Jaffrezic, C. Martelet and S. Cosnier, A laponite clay-poly(pyrrole–pyridinium) matrix for the fabrication of conductimetric microbiosensors, *Anal. Chim. Acta* 401 (1999) 117–124.

[77] H. Barhoumi, A. Maaref, S. Cosnier, C. Martelet and N. Jaffrezic-Renault, Urease immobilization on biotinylated polypyrrole coated ChemFEC devices for urea biosensor development, *IRBM* 29 (2008) 192-201.

[78] T. Ahuja, I.A. Mir, D. Kumar and R. Rajesh, Potentiometric urea biosensor based on BSA embedded surface modified polypyrrole film, *Sens. Actuators B* 134 (2008) 140-145.

[79] S. Bourigua, I. Hafaid, H. Korri-Youssoufi, A. Maaref and N. Jafferezic-Renault, A Novel Urea Biosensor Based on Modified Electrodes with Urease Immobilized on Poly(N-hydroxyphtalimide-pyrrole-co-pyrr-ole) Film Incorporating Ethyl Amine Ferrocene as Redox Marker, *Sens. Lett.* 7 (2009) 731-738.

[80] I. Bozgeyik, M. Senel, E. Cevik and M.F. Abasiyanik, A novel thin film amperometric urea biosensor based on urease-immobilized

on poly(N-glycidylpyrrole-co-pyrrole), *Curr. Appl. Phys.* 11 (2011) 1083-1088.

[81] W.Z. Jia, L. Su and Y. Lei, Pt nanoflower/polyaniline composite nanofibers based urea biosensor, *Biosens. Bioelectron.* 30 (2011) 158-164.

[82] S. Suman E. O'Reilly, M. Kelly, A. Morrin, M.R. Smyth and A.J. Killard, Chronocoulometric determination of urea in human serum using an inkjet printed biosensor, *Anal. Chim. Acta* 697 (2011) 98-102.

[83] B. Lakard, D. Magnin, O. Deschaume, G. Vanlancker, M. Glinel, S. Demoustier-Champagne, B. Nysten, A.M. Jonas, P. Bertrand and S. Yunus, Urea potentiometric enzymatic biosensor based on charged biopolymers and electrodeposited polyaniline, *Biosens. Bioelectron.* 26 (2011) 4139-4145.

[84] S.K. Jha, M. Kanungo, A. Nath and S.F. D'Souza, Entrapment of live microbial cells in electropolymerized polyaniline and their use as urea biosensor, *Biosens. Bioelectron.* 24 (2009) 2637-2642.

[85] S. Uchiyama, M. Kobayashi and Y. Hasebe, Electrical control of urease activity immobilized to the conducting polymer on the carbon felt electrode, *Electroanalysis* 14 (2002) 1644-1647.

[86] P.C. Pandey and G. Singh, Tetraphenylborate doped polyaniline based novel pH sensor and solid-state urea biosensor, *Talanta* 55 (2001) 773–782.

[87] M.M. Castillo-Ortega, D.E. Rodriguez, J.C. Encinas, M. Plascencia, F.A.M. Velarde and R. Olayo, Conductometric uric acid and urea biosensor prepared from electroconductive polyaniline–poly(n-butyl methacrylate) composites, *Sens. Actuators B* 85 (2002) 19-25.

[88] P.C. Pandey, S. Upadhyay, G. Singh, R. Prakash, R.C. Srivastava and P.K. Seth, A new solid-state pH sensor and its application in the construction of all solid-state urea biosensor, *Electroanalysis* 12 (2000) 517-521.

[89] S.B. Adeloju, S.J. Shaw and G.G. Wallace, Polypyrrole-based potentiometric biosensor for urea Part 1. Incorporation of urease, *Anal. Chim. Acta* 281 (1393) 611-620.

[90] S.B. Adeloju, S.J. Shaw and G.G. Wallace, Polypyrrole-based potentiometric biosensor for urea: Part 2. Analytical optimisation, *Anal. Chim. Acta* 281 (1993) 621-627.

[91] S. Komaba, M. Seyama, T. Momma and T. Osaka, Urea biosensor based on the composite film of electroinactive polypyrrole and urease modified with polyanion, *Denki Kagaku* 64 (1996) 1228-1233.

[92] J.M.C.S. Magalhaes and A.A.S.C. Machado, Urea potentiometric biosensor based on urease immobilized on chitosan membranes, *Talanta* 47 (1998) 183-191.

[93] B. Lakard, G. Herlem and S. Lakard, Urea potentiometric biosensor based on modified electrodes with urease immobilized on polyethylenimine films, *Biosens. Bioelectron.* 19 (2004) 1641-1647.

[94] R. Koncki, A. Hulanicki, S. Glab, Biochemical modifications of membrane ion-selective sensors, *Trends Anal. Chem.* 76 (1997) 528-536.

[95] R. Koncki, A. Radomska and S. Glab, Potentiometric determination of dialysate urea nitrogen, *Talanta* 52 (2000) 13-17.

[96] R. Koncki, A. Radomska and S. Glab, Bioanalytical flow-injection system for control of hemodialysis adequacy, *Anal. Chem. Acta* 418 (2000) 213-224.

[97] R. Koncki, G.J. Mohr and O.S. Wolfbeis, Enzyme biosensor for urea based on a novel pH bulk optode membrane, *Biosens. Bioelectron.* 10 (1995) 653-659.

[98] R. Koncki, A. Chudzik and I. Walcerz, Urea determination using pH–enzyme electrode, *J. Pharm. Biomed. Anal.* 21 (1999) 51–57.

[99] B. Kovacs, G. Nagy, R. Dombi and K. Toth, Optical biosensor for urea with improved response time, *Biosens. Bioelectron.* 18 (2003) 111-118.

[100] K. Sawicka, P. Gouma and S. Simon, Electrospun biocomposite nanofibers for urea biosensing, *Sens. Actuators B* 108 (2005) 585-588.

[101] R. Singhal, A. Gambhir, M.K. Pandey, S. Annapoorni and B.D. Malhotra, Immobilization of urease on poly(N-vinyl carbazole) /stearic acid Langmuir/Blodgett films for application to urea biosensor, *Biosens. Bioelectron.* 17 (2002) 697-703.

[102] V. Rebriiev and N.F. Starodub, Enzymatic biosensor based on the ISFET and photopolymeric membrane for the determinaion of urea, *Electroanalysis* 16 (2004) 1891-1895.

[103] W. Limbut, P. Thavarungkul, P. Kanatharana, P. Asawatreratanakul, C. Limsakul and B. Wongkittisuksa, Comparative study of controlled pore glass, silica gel and Poraver® for the immobilization of urease to determine urea in a flow injection conductimetric biosensor system, *Biosens. Bioelectron.* 19 (2004) 813–821.

[104] Y. Xu, C. Lu, Y. Hu, L. Nie and S. Yao, Preparation of urea sensors with a series

piezoelectric crystal device, Anal. Lett. 29 (1996) 1069-1080.

[105] F. Kuralay, H. Ozyoruk and A. Yıldız, Amperometric enzyme electrode for urea determination using immobilized urease in poly(vinylferrocenium) film, *Sens. Actuators B* 114 (2006) 500–506.

[106] S. Milardovic, I. Kruhak and B.S. Grabaric, Urea determination in FIA mode by a newly designed urease-based biosensor, *LRA* 11 (1999) 266–271.

[107] J.V. Melo, S. Cosnier, C. Mousty, C. Martelet and N. Jaffrezic-Renault, Urea biosensors based on immobilization of urease into two oppositely charged clays (Laponite and Zn-Al layered double hydroxides), *Anal. Chem.* 74 (2002) 4037-4043.

[108] S.M. Macha and A. Fitch, Clays as architectural units at modified-electrodes, *Mikrochim. Acta* 128 (1998) 1-18.

[109] S. Vial, C. Forano, D. Shan, C. Mousty, H. Barhoumi, C. Martelet and N. Jaffrezic, Nanohybrid-layered double hydroxides/ urease materials: Synthesis and application to urea biosensors, *Mater. Sci. Eng. C* 26 (2006) 387–393.

[110] B. Liu, R. Hub and J. Dengay, Studies on a potentiometric urea biosensor based on an ammonia electrode and urease, immobilized on a y-aluminum oxide matrix, *Anal. Chim. Acta* 341 (1997) 161-169.

[111] W. Limbut, P. Thavarungkul, P. Kanatharana, P. Asawatreratanakul, C. Limsakul and B. Wongkittisuksa, Comparative study of controlled pore glass, silica gel and Poraver® for the immobilization of urease to determine urea in a flow injection conductimetric biosensor system, *Biosens. Bioelectron*. 19 (2004) 813–821.

[112] L. Tymecki, E. Zwierkowska and R. Koncki, Strip bioelectrochemical cell for potentiometric measurements fabricated by screen-printing, *Anal. Chem. Acta* 538 (2005) 251-256.

[113] L. Tymecki and R. Koncki, Thick-film potentiometric biosensor for bloodless monitoring of hemodialysis, *Sens. Actuators B* 113 (2006) 782-786.

[114] O. Lev, Z. Wu, S. Bharathi, V. Glezer, A. Modestov, J. Gun, L. Rabinovich and S. Sampath, Sol-gel materials in electrochemistry, *Chem. Mater.* 9 (1997) 2354-2375.

[115] N.F. Sheppard and D.J. Mears, Model of an immobilized enzyme conductimetric urea biosensor, *Biosens. Bioelectron.* 11 (1996) 967-979.

[116] A. Olatowska-Jarza, H. Podbielska, Influence of organic dopant-urease on sol-gel matrix optical properties in visible range, *Optica Applicata* 30 (2000) 193-197.

[117] D.D. Cunningham, Construction of a potentiometric biosensor for monitoring urea during peritoneal dialysis, *Sens. Lett.* 2 (2004) 217-220.

[118] D.G. Pijanowska and W. Torbicz, pH-ISFET based urea biosensor, *Sens. Actuators B* 44 (1997) 370-376.

[119] W. Qin, Z. Zhang and Y. Peng, Plant tissue-based chemiluminescence flow biosensor for urea, *Anal. Chim. Acta* 407 (2000) 81-86.

[120] B. Bjarnason, P. Johansson and G. Johansson, A novel thermal biosensor: evaluation for determination of urea in serum, *Anal. Chim. Acta* 372 (1998) 341-348.

[121] B. Xie and B. Danielsson, An integrated thermal biosensor array for multianalyte determination demonstrated with glucose, urea and penicillin, *Anal. Lett.* 29 (1996) 1921-1932.

[122] B. Xie, M. Mecklenburg, B. Danielsson, O. Ohman, P. Norlin and F. Winquist, Development of an integrated thermal biosensor for the simultaneous determination of multiple analytes, *Analyst* 120 (1995) 155-160.

[123] L.D. Ciana and G. Caputo, Robust, reliable biosensor for continuous monitoring of urea during dialysis, *Clin. Chem.* 42 (1996) 1079-1085.

[124] S. Zamponi, B.L. Cicero, M. Mascini, L.D. Ciana and S. Sacco, Urea solid-state biosensor suitable for continuous dialysis control, *Talanta* 43 (1996) 1373-1377.

[125] P.K. Srivastava and A.M. Kayastha, Characterization of gelatin-immobilized pigeonpea urease and preparation of a new urea biosensor, *Biotechnol. Appl. Biochem.* 34 (2001) 55-62.

[126] R. Koncki, I. Walcerz and E. Leszczynska, Enzymatically modified ion-selective electrodes for flow injection analysis, *J. Pharm. Biomed. Anal.* 19 (1999) 633-638.

[127] J.D. Gracia, M. Poch, D. Martorell and S. Alegret, Use of mathematical models to describe dynamic behaviour of potentiometric biosensors: Comparison of deterministic and empirical approaches to an urea flow-through biosensor, *Biosens. Bioelectron.* 11 (1996) 53-61.

[128] X. Wang, H. Watanabe, N. Sekioka, H. Hamana and S. Uchiyama, Amperometric urea biosensor using aminated glassy carbon electrode covered with urease immobilized carbon sheet, based on the electrode oxidation of carbamic acid, *Electroanalysis*

19 (2007) 1300-1306.
[129] S.K. Jha, A. Topkar and S.F. D'Souza, Development of potentiometric urea biosensor based on urease immobilized in PVA-PAA composite matrix for estimation of blood urea nitrogen (BUN), *J. Biochem. Biophys. Methods* 70 (2008) 1145-1150.
[130] N.H. Chou, J.C. Chou, T.P. Sun and S.K. Hsiung, Flexible SnO_2 Substrate-Based Potentiometric Thick-Film Solid-State Urea Biosensor, *Sens. Lett.* 6 (2008) 831-835.
[131] U.B. Trivedi, D. Lakshminarayana, I.L. Kothari, N.G. Patel, H.N. Kapse, K.K Makhija, R.B. Patel and C.J. Panchal, Potentiometric biosensor for urea determination in milk, *Sens. Actuators B* 140 (2009) 260-266.
[132] E. Tsutsumi, T.G. Henares, K. Kawamura, T. Yao and H. Hisamoto, Facile Preparation Method of a Disposable Capillary Biosensor Using an Ion-selective Optode Membrane and a Dissolvable Enzyme Membrane and Its Application to Urea Sensing, *Chem. Lett*. 39 (2010) 436-438.
[133] A.R. Barbosa, A. Karmali, Development of a biosensor for urea assay based on amidase inhibition, using an ion-selective electrode, *Biocatal. Biotransfor.* 29 (2011) 130-140.
[134] A. Ulianas, L.Y. Heng, M. Ahmad, A Biosensor for Urea from Succinimide-Modified Acrylic Microspheres Based on Reflectance Transduction, *Sensors* 11 (2011) 8323-8338.
[135] D. Chirizzi and C. Malitesta, Potentiometric urea biosensor based on urease immobilized by an electrosynthesized poly(o-phenylene-diamine) film with buffering capability, *Sens. Actuators B* 157 (2011) 211-215.
[136] B. Dindar, E. Karakus and F. Abasiyanik, New Urea Biosensor Based on Urease Enzyme Obtained from Helycobacter pylori, *Appl. Biochem. Biotechnol.* 165 (2011) 1308-1321.
[137] H. Barhoumi, A. Maaref, M. Rammah, C. Martelet, N. Jaffrezic, C. Mousty, S. Vial and C. Forano, Urea biosensor based on Zn_3Al–Urease layered double hydroxides nanohybrid coated on insulated silicon structures, *Mater. Sci. Eng. C* 26 (2006) 328 – 333.
[138] A. Seki, S. Ikeda, I. Kubo and I. Karube, Biosensors based on light-addressable potentiometric sensors for urea, penicillin and glucose, *Anal. Chim. Acta* 373 (1998) 9-13.
[139] X. Chen, Z.P. Yang and S.H. Si, Potentiometric urea biosensor based on immobilization of urease onto molecularly imprinted TiO_2 film, *J. Electroanal. Chem.* 635 (2009) 1-6.
[140] T.M. Pan, M.D. Huang, W.Y. Lin and M.H. Wu, A urea biosensor based on pH-sensitive Sm2TiO5 electrolyte-insulator-semiconductor, *Anal. Chim. Acta* 669 (2010) 68-74.
[141] G. Sumana, M. Das, S. Srivastava and B.D. Malhotra, A novel urea biosensor based on zirconia, *Thin Solid Films* 519 (2010) 1187-1191.
[142] S.K. Kirdeciler, E. Soy, S. Ozturk, I. Kucherenko, O. Soldatkin, S. Dzyadevych and B. Akata, A novel urea conductometric biosensor based on zeolite immobilized urease, *Talanta* 85 (2011) 1435-1441.
[143] G.K. Mishra, R.K. Mishra and S. Bhand, Flow injection analysis biosensor for urea analysis in adulterated milk using enzyme thermistor, *Biosens. Bioelectron.* 26 (2010) 1560-1564.
[144] E. Cevik, M. Senel and M.F. Abasiyanik, An amperometric urea biosensor based on covalent immobilization of urease on copolymer of glycidyl methacrylate and vinylferrocene, *J. Solid State Electrochem.* 16 (2012) 367-373.
[145] M.S. Alqasaimeh, L.Y. Heng and M. Ahmad, A urea biosensor from stacked sol-gel films with immobilized nile blue chromoionophore and urease enzyme, *Sensors* 7 (2007) 2251-2262.
[146] B. Premanode and C. Toumazou, A novel, low power biosensor for real time monitoring of creatinine and urea in peritoneal dialysis, *Sens. Actuators B* 120 (2007) 732-735.
[147] Z.P. Yang, S.H. Si, H.J. Dai and C.J. Zhang, Piezoelectric urea biosensor based on immobilization of urease onto nanoporous alumina membranes, *Biosens. Bioelectron.* 22 (2007) 3283-3287.
[148] A. Tiwari, S. Aryal, S. Pilla and S.Q. Gong, An amperometric urea biosensor based on covalently immobilized urease on an electrode made of hyperbranched polyester functionalized gold nanoparticles, *Talanta* 78 (2009) 1401-1407.
[149] M. Swati, N.K. Hase and R. Srivastava, Nanoengineered optical urea biosensor for estimating hemodialysis parameters in spent dialysate, *Anal. Chim. Acta* 676 (2010) 68-74.
[150] T. Ahuja, D. Kumar, N. Singh, A.M. Biradar and R. Rajesh, Potentiometric urea biosensor based on multi-walled carbon nanotubes (MWCNTs)/silica composite material, *Mater. Sci. Eng. C Mater. Biol. Appl.* 31 (2011) 90-94.
[151] K. Gabrovska, J. Ivanov, I. Vasileva, N. Dimova and T. Godjevargova, Immobili-

zation of urease on nanostructured polymer membrane and preparation of urea amperemetric biosensor, *Int. J. Biol. Macromol.* 48 (2011) 620-626.

[152] N. Palomera, M. Balaguera, S.K. Arya, S. Hernandez, M.S. Tomar, J.E. Ramirez-Vick and S.P. Singh, Zinc Oxide Nanorods Modified Indium Tin Oxide Surface for Amperometric Urea Biosensor, *J. Nanosci. Nanotech.* 11 (2011) 6683-6689.

[153] O.O. Soldatkin, E. Soy, A. Errachid, N. Jaffrezic-Renault, B. Akata, A.P. Soldatkin and S.V. Dzyadevych, Influence of Composition of Zeolite/Enzyme Nanobiocomposites on Analytical Characteristics of Urea Biosensor Based on Ion-Selective Field-Effect Transistors, *Sens. Lett.* 9 (2011) 2320-2326.

[154] Y. Sahraoui, H. Barhoumi, A. Maaref and J.R. Nicole, A Novel Capacitive Biosensor for Urea Assay Based on Modified Magnetic Nanobeads, SENSOR LETTERS 9 (2011) 2141-2146.

[155] S. Bourigua, A. Errachid, S. Dzyadevych, A. Maaref and N.J. Renault, Impedimetric Urea Biosensor Based on Single-Wall Carbon Nanotubes (SWNCT-COOH) and Polypyrrole, SENSOR LETTERS 9 (2011) 2232-2235.

[156] W. Nouira, A. Maaref, F. Vocanson, M. Siadat, J. Saulnier, F. Lagarde and N. Jaffrezic-Renault, Enhancement of Enzymatic IDE Biosensor Response Using Gold Nanoparticles. Example of the Detection of Urea, ELECTROANALYSIS 24 (2012) 1088-1094.

[157] F. Branzoi, V. Branzoi and A. Musina, Amperometric urea biosensor based on platinum electrode modified with a nanocomposite film, SURFACE AND INTERFACE ANALYSIS 44 (2012) 895-898.

[158] D. Yun, M.J. Song, S. Hwang and S.I. Hong, Fabrication and Electrochemical Characterization of Nanoporous Silicon Electrode for Amperometric Urea Biosensor, JAPANESE JOURNAL OF APPLIED PHYSICS 51 (2012)

[159] S. Srivastava, M.A. Ali, P.R. Solanki, P.M. Chavhan, M.K. Pandey, A. Mulchandani, A. Srivastava and B.D. Malhotra, Mediator-free microfluidics biosensor based on titania-zirconia nanocomposite for urea detection, RSC ADVANCES 3 (2013) 228-235.

[160] E. Cevik, M. Senel and A. Baykal, Potentiometric urea biosensor based on poly(glycidylmethacrylate)-grafted iron oxide nanoparticles, CURRENT APPLIED PHYSICS 13 (2013) 280-286.

[161] M. Tyagi, M. Tomar and V. Gupta, NiO nanoparticle-based urea biosensor, Biosens. Bioelectron. 41 (2013) 110-115

[162] H. Barhoumi, A. Maaref and N. Jaffrezic-Renault, Urea Impedimetric Biosensor Based on Modified Gold Microelectrode with 2-Amino-6-Purinethiol, SENSOR LETTERS 9 (2011) 2116-2122.

[163] C.Y. Pang, Y.C. Zhu, H.Y. Gao, Y. Dong and J. Lu, A novel urea amperometric biosensor based on secretion of carnation petal cells modified on a graphite-epoxy composite electrode, ANALYST 136 (2011) 841-846.

[164] K.M. Chang, C.T. Chang and K.M. Chan, Development of an Ion Sensitive Field Effect Transistor Based Urea Biosensor with Solid State Reference Systems, Sens. 10 (2010) 6115-6127.

[165] B. Premanode and C. Toumazou, A novel, low power biosensor for real time monitoring of creatinine and urea in peritoneal dialysis, *Sens. Actuators B* 120 (2007) 732-735.