ION-SELECTIVE ELECTRODE AND ENZYME SENSORS FOR FLOW-TYPE ENVIRONMENTAL ANALYSIS

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Dedicated to the memory of Prof. J. Heyrovský on the occasion of his centenary.

Illustrative examples from researches in the author's laboratories are given of potentiometric ion-selective electrodes (ISEs) and catalytic membrane amperometric electrodes suitable for environmental analysis. The uses of sulphide ISEs are demonstrated for monitoring the activities of sulphate-reducing bacteria, in effluent analysis for sulphide, and in process/effluent analysis for sulphide, thiols and polysulphides. Diquat and paraquat ISEs are discussed in relation to their optimisation in terms of appropriate crown ether and ion-pairing agents, together with prospects of their uses. Catalytic membrane systems for use in electrochemical analysis may be based on metal oxide catalysts or enzymes. The best of the new metal oxide catalysts for hydrogen peroxide analysis in association with an oxygen electrode is based on manganese dioxide and offers an alternative to lead dioxide and catalase. An alternative membrane system for use with a platinum electrode is peroxidase in association with a mediator in order to permit a low potential approach for hydrogen peroxide sensing. Hydrogen peroxide is a product of many oxidase catalysed reactions and its electrochemical detection permits the analysis of a wide range of substrates, ilustrated here for glucose in various food products and hypoxanthine in the spoilage of fish meats.

Essentially, the main objectives of environmental chemistry are an appreciation and understanding of the nature, distribution and fate in the environment of those compounds that affect the quality of life. Analytical chemistry plays an important part in the achievement of these objectives. There is, therefore, a constant need for new analytical approaches, as well as more sensitive methods in order to cope with the increasing demands in the field. Furthermore, the formulation of analytical methods for pollutants has gone hand-in-glove with new regulations for controlling and monitoring the release of pollutants into the environment.

Electrochemical methods are among the several analytical methodologies currently in use, and new developments point to new approaches. Ion-selective electrodes and enzyme electrodes are among newer electrochemical approaches available to the field, and it is the aim of this paper to illustrate some developments and applications of these devices from the research studies within the author's laboratories. Thus, the

illustrations will be based on the solid state membrane sulphide and the PVC matrix membrane diquat and paraquat systems from among potentiometric ion-selective electrodes (ISEs), and enzyme and metal oxide membranes with amperometric electrodes for catalytic membrane systems in electrochemical sensing.

POTENTIOMETRIC ION-SELECTIVE ELECTRODES

ISEs are widely used in environmental type analysis and, among these, the sulphide ISE has interesting rôles in monitoring the sulphate-reducing bacteria, in the analysis of sulphide in effluents, and for the differential analysis of sulphides, thiols and poly-sulphides in process and effluent streams. Such uses of the sulphide ISE are promoted by its utility over a wide range of concentration[1].

Sulphate Reducing Bacteria

Anaerobic corrosion by sulphate-reducing bacteria has expensive economic and technological consequences. They corrode buried pipes, spoil petrol and cutting oil emulsion, corrode oilwell casings, blacken paper pulp, and produce hydrogen sulphide in natural and manufactured gases[2].

Since hydrogen sulphide is a product of the metabolic activities of sulphate--reducing bacteria, trapping of this in sodium hydroxide can yield useful informa-tion. A convenient way is to set up the bacteria in a nutrient consisting of the sulphate or other sulphur-based substrate with an organic nutrient, such as lactic acid, in a culture flask. The hydrogen sulphide product is swept into a monitoring flask fitted with a sulphide/reference electrode pair and charged with 1M sodium hydroxide. In this way, it has been shown[3] that metal sulphate is quantitatively metabolised to sulphide by various *Desulfovibrio* species, including *desulfuricans, gigas* and *vul-garis*[3,4]. Older bacteria require a lag phase, while fresh bacteria activate quickly. Furthermore, the bacteria will metabolise various intermediates, such as sulphite, thiosulphate, metabisulphite and dithionite[3,4]. Interestingly, *D. vulgaris* has been shown to grow in certain organic sulphur species, e.g., cysteine, cystine and gluta-thione as alternative sulphur species to sulphate[4]. Such growth is linked to the presence of $-S-C-C-NH_2$ links, which are absent in methionine (this has a $C-S-C$ linkage) and in which the bacteria do not grow[4].

Sulphide in Effluents and Process Streams

Flow injection analysis (FIA) is a convenient approach to quickly analysing a suc-cession of samples of similar origin, by injecting samples into a carrier stream, carrying out any necessary chemistry (if necessary) and recording the response of the sensor placed downstream. Electrochemical sensors, including ISEs, lend them-

selves admirably to this approach. ISEs can be used in various modes, including the cascade and flow-through approaches. Such approaches promote the ability to use sulphide ISEs to monitor sulphide in sewage effluents before and after treatment with hydrogen peroxide. The ability to do this is important in view of the scope of hydrogen peroxide for treating and controlling deleterious sulphide types in effluents, including sewage effluents[5].

An assessment of the merit of high strength Sulphide Anti-Oxidant Buffer (SAOB) (2M sodium hydroxide plus 40 g l^{-1} ascorbic acid) is shown in Table I for sewage effluent samples[6]. Remembering that the colorimetric sulphide levels of samples 4 and 7 are near to the background level (5 μM = 0·16 p.p.m.) of sulphide in the FIA carrier stream, and which really excludes them from the discussion, the FIA data by both "Cascade" and "flow-through" ISEs for the remaining samples are close to the colorimetric data. Thus, FIA in either mode, following sample treatment with the high-strength SAOB (sample diluted 1 + 1 with SAOB containing 4M sodium hydroxide and 80 g l^{-1} ascorbic acid), is suitable for control monitoring

TABLE I

Sulphide content of sewage effluents following storage for 3 days in sealed containers determined by colorimetry, direct potentiometry and FIA[a] (data from ref.[5]). S.ds in parentheses for $n = 4$

| Sample No. | Sulphide content, mg l^{-1} | | | |
| | Colorimetry | Direct potentiometry | FIA | |
			EDT EES[c]	Orion[d]
1	2·1 (0·2)	1·5 (0·2)	2·1 (0·3)	—
2	15·2 (0·8)	11·1 (0·2)	15·0 (2·0)	—
3	11·6 (0·3)	14·8 (2·0)	12·8 (0·1)	—
4[b]	0·20 (0·1)	0·20 (0·0)	0·22 (0·55)	—
Mean	*7·28*	*6·90*	*7·52*	
5	17·8 (0·5)	14·5 (1·2)	—	17·9 (0·1)
6	16·5 (1·1)	13·2 (0·1)	—	16·7 (0·9)
7[b]	0·10 (0·05)	0·12 (0·05)	—	0·45 (0·07)
8[b]	2·4 (1·1)	1·3 (0·8)	—	2·0 (1·0)
Mean	*9·0*	*7·28*		*9·26*

[a] For FIA the carrier stream was 5 . 10^{-6}M sodium sulphide in normal SAOB and samples were diluted (1 + 1) with SAOB containing ascorbic acid (80 g l^{-1}) in 4M sodium hydroxide; [b] sewage effluent treated with hydrogen peroxide; [c] EDT EES flow-through sulphide electrode; [d] Orion 94 16A "cascade flow" sulphide ISE.

of sulphide in sewage effluent. Such monitoring can also be made on hydrogen peroxide treated samples (4, 7 and 8) when the aim is to ensure that sulphide is kept below a threshold value[5].

As mentioned above, the sulphide ISE will respond to sulphide and thiols, but is much more sensitive to sulphide. This has been put to advantage in a computerised titration system in petroleum process streams and effluents, and which gave an intermediate inflexion to polysulphide[6]. Thus, from the second derivative of a titration graph (with 10^{-3}M silver nitrate titrant) for a spent caustic soda oil refinery sample, titration end-points of 4·50, 5·33 and 9·56 cm^3 were obtained on a diluted 0·5 cm^3 sample and corresponding to the end-points for sulphide, polysulphide and thiol[6].

Diquat and Paraquat Sensing

Various PVC matrix membrane ISE types have been studied[7] for diquat and paraquat, initially based on dibenzo-30-crown-10 (DB30C10) as sensor, and with membrane compositions shown in Table II. The selection of the DB30C10 crown ether was based on the maximum stability[8-10] of its complex with DQT compared to that of other crown ethers (Table III), facilitated by three DB30C10 (host)-DQT (guest) interactions, namely: (i) DB30C10 catechol–oxygen electrostatic interaction with the positively charged nitrogen atoms in DQT. For this, the crown ether catechol O–O separation (2·6 Å) and N–N separation in DQT (2·8 Å) are similar so that the former are nearly directly above and below the latter in the $[DQT.DBC10]^{2+}$ complex; (ii) DB30C10 benzene ring π-electron charge transfer to the electron deficient DQT^+; (iii) hydrogen bonding between H^6 and $H^{6'}$ with oxygen atom in the DB30C10 framework.

Type 1 electrodes tended to be more unstable[7]. Selectivity for DQT over other cations is much improved in Type 2 electrodes. Type 3 electrodes, consisting of DQT.2TPB with appropriate solvent mediators, also responded well[7] (Table IV). For both Types 2 and 3, the most appropriate solvent mediators[7] are either 2-nitrophenyl phenyl ether (NPPE) or 2-nitrophenyl octyl ether (NPOE). Selectivity coefficients ($k_{DQT,B}^{pot}$) have been determined[7] for B = Li, Ba, K, Mg, Ca, Ba, ammonium, diethylammonium, anilinium and guanidinium. Although these frequently exceed unity for B^{n+} species for $n = 1$, the modifying effect of the square power term ($n+ = 2+$ for DQT) in the selectivity relation[11] enhances the practical selectivity towards DQT^7.

In the corresponding study[7] on PQT, Type 3 electrodes were found to be of better quality than Type 2. This difference may be related to the fact that DB30C10 changes its shape dramatically to accommodate its DQT guest molecules but does not accommodate PQT molecules[11]. On the other hand, bis-paraphenylene-34-crown-10 (BPP34C10) engulfs PQT and hardly alters in shape[11]. This feature was planned to

be exploited if the further study[12] on a wider range of crown ethers in association with dibutyl phthalate (DBP) and 2-nitrophenyl octyl ether (NPOE) as plasticising solvent mediators showed this feature to promote good ISE properties for PQT.

It was not possible to distinguish between DB30C10, bis-metaphenylene-32-crown-10 (BMP32C10), bis-metaphenylene-38-crown-12 (BMP38C12), BPP34C10, bis-paraphenylene-37-crown-11 (BPP37C11) and dinaphthalene-36-crown-10 (DN36-C10) for electrodes based on 2-nitrophenyl octyl ether solvent mediator and treated with PQT dichloride, since even the electrodes with PVC membranes containing NPOE alone responded strongly to PQT[12]. The DBP-based electrodes gave relatively low PQT response. These studies[12] suggest weak PQT complexation by the crown ethers, and attributable to two effects, namely, the N–N separation in PQT (7·0 Å) is dissimilar to the separations between the oxygens linked to the aromatic rings (unlike the case of DQT discussed above for DB30C10). Also, there is a reduced possibility for hydrogen bonding through the CH_3 groups attached to the PQT nitrogens compared with, say, 4,4′-dipyridyl for which there is good ISE function[12].

TABLE II
Composition of PVC matrix membrane types used for electrode construction (from ref.[12])

| Membrane | Membrane composition, % mass ratio | | | |
	Solvent mediator	PVC	Crown ether e.g., DB30C10	DQT.2 TPB or PQT.2 TPB
1	67·0	31·6	1·4	—
2	62·3	29·4	1·3	7·0
3	63·0	30·0	—	7·0

TABLE III
Stability constants (K_a) and free energies of formation (ΔG_f) of [DQT.DB3nCrown-n] complexes in acetone (data from refs[8−10])

Crown ether	K_a, mol^{-1} l	ΔG_f, kJ mol^{-1}
DB27C9	$4·1 . 10^2$	15·0
DB30C10	$1·8 . 10^4$	24·3
DB33C11	$1·1 . 10^4$	23·1
DB36C12	$2·0 . 10^3$	18·9
DB30C10(−OCH$_2$)$_2$Ph	—	31·0

TABLE IV
Response characteristics of ISEs for DQT. S.ds in parentheses are for $n = 5-7$, spread over 7d (from ref.[12])

| Solvent mediator[a]	Type 1				Type 2				Type 3			
	S mV	C mV	$a_{min}\cdot 10^6$ mol l^{-1}	r	S mV	C mV	$a_{min}\cdot 10^6$ mol l^{-1}	r	S mV	C mV	$a_{min}\cdot 10^6$ mol l^{-1}	r
DBP	35·6 (3·3)	179 (15·7)	9·7 (2·2)	0·991	25·2 (0·35)	135 (3·1)	4·2 (0·4)	0·998	34·1 (0·6)	186 (5·3)	3·5 (0·8)	0·998
DNP	b	b	b	b	17·5 (1·1)	8·1 (1·6)	24 (11)	0·89	27·3 (1·2)	114 (10)	430 (360)	0·987
DOPP	47·0[c]	233	11	0·998	21·8 (0·2)	105 (2·0)	16 (2)	0·997	27·0 (2·2)	149 (3·4)	3·3 (2)	0·997
NPOE	32·6 (2·1)	151 (18·7)	31 (24)	0·989	28·8 (0·6)	170 (6·4)	1·5 (1·1)	0·999	31·2 (1·9)	160 (27)	18 (20)	0·998
NPPE	b	b	b	b	29·8 (1·4)	169 (11)	1·4 (0·4)	·0·999	26·7 (0·9)	153 (5·3)	1·8 (0·4)	0·999

[a] DBP dibutyl phthalate, DNP dinonyl phthalate, DOPP dioctyl phenylphosphonate, NPOE o-nitrophenyl octyl ether, and NPPE o-nitrophenyl phenyl ether; [b] functional electrodes not obtained; [c] electrode slope unstable.

The weakness of the crown ether: PQT stability constants compared with that for $(DB30C10.DQT)^{2+}$ $(K_a = 1.8 \cdot 10^4 \text{ mol } l^{-1}$ as given in Table III) is notable. Thus, the K_a data for $(BMP32C10\cdot PQT)^{2+}$ and $(BPP34C10.PQT)^{2+}$ are just 760 mol l^{-1} and 730 mol l^{-1}, respectively, and pointing to insufficient affinity by the crown ethers to ensure adequate ISE sensing[12].

As suggested above, functional DQT and PQT ISEs derive from the simple salts with counter anions, such as tetraphenylborate (TPB) or tetra-4-chlorophenylborate (T4C1PB). Such a system in PVC with 2-nitrophenyl phenyl ether solvent mediator yields an ISE for DQT that was stable for 55 days[13]. Samples $(2.54-244 \text{ μM DQT})$ could be determined[13] using the standard addition method with about a -5% error and a precision of 7 to 8%. For comparison, DQT was also determined[13] by titration with sodium tetraphenylborate, when the errors were 12 to 20% for DQT analysis in deionised water, sodium chloride solution or simulated serum.

AMPEROMETRIC ELECTRODES WITH CATALYTIC MEMBRANES

The substrates and/or products of many enzyme-catalysed reactions are either electrochemically active, or can be made to be so by suitable reactions. This provides many opportunities for the design of selective electrodes for use in analysis. For example, the glucose oxidase catalysed oxidation of glucose

$$\text{glucose } + \text{ O}_2 \xrightarrow[\text{oxidase}]{\text{glucose}} \text{ gluconic acid } + \text{ H}_2\text{O}_2 \qquad (1)$$
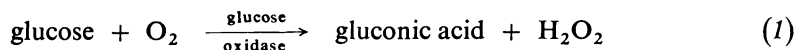
permits the determination of glucose from an amperometric type signal pertaining to the consumption of oxygen or the production of hydrogen peroxide. The enzyme is best immobilised on a membrane covering the indicating electrode to give an enzyme electrode. Although the enzyme can be physically immobilised on a support matrix, a convenient, more lasting approach is chemical immobilisation by linking the enzyme through the agency of glutaraldehyde via lysine spacer attached to preactivated nylon net[14,15].

Reaction (1) highlights the fact that hydrogen peroxide can be amperometrically monitored. This is noteworthy, for hydrogen peroxide itself is an important material whose main uses are in the maintenance of a better environment and of improving the quality of life. Thus, it is used for wastewater treatment, sterilisation, and as a source of oxygen. It also crops up in life processes, e.g., it is a principal product of the oxidation of various biochemical materials as in reactions catalysed by enzymes as illustrated by reaction (1).

Application of the platinum electrode for determining the hydrogen peroxide produced during enzyme reactions has been perfected for the FIA of enzymatically produced hydrogen peroxide at such an electrode operated at $+600 \text{ mV}$ vs a silver--silver chloride reference electrode[15]. An illustration of a practical application is

the determination of the glucose content of food materials, such as icecream and other glucose-containing foods[15].

Avoidance of Interferences

Use of the platinum electrode at +600 V vs the reference electrode as above is frequently fraught with interferences due to concurrent oxidation of other materials in the sample matrix. Thus, among the alternative approaches, Gorton[16] has used a carbon electrode sputtered with palladium and gold. Here, anodic oxidation occurs at a lower potential of +400 mV and cathodic reduction at −150 mV vs the SCE. Certain interferences may also be avoided by measuring the oxygen produced by the catalytical decomposition of hydrogen peroxide. Thus, metal oxide membranes[17,18] and catalase immobilised on collagen[19], and cellulose acetate[20] or catalase-rich bovine liver slices[21] have been used.

Another method of avoiding interferences is to further exploit the use of low potential by employing a soluble mediator, such as hexacyanoferrate(II), in the presence of peroxidase as a catalyst:

$$H_2O_2 + 2\,[Fe(CN)_6]^{4-} + 2\,H^+ \xrightarrow{\text{peroxidase}} 2\,H_2O + 2\,[Fe(CN)_6]^{3-} . \quad (2)$$
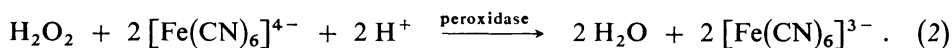
The mediation is by the hexacyanoferrate(III) being then reduced at a lower potential (−100 mV) than would normally be the case for direct hydrogen peroxide detection. The method is illustrated by the determination of hypoxanthine[22], which can be related to monitoring fish meat spoilage (Table V). A variant of this is the use of immobilised peroxidase with immobilised electron transfer reagents[23].

TABLE V

Hypoxanthine content of fish meat determined with a xanthine oxidase/peroxidase electrode (BE) and by spectrophotometry (AMC) (data from ref.[22])

	Hypoxanthine, μmol g^{-1}			
	fresh fish		after 20 h storage, 20°C	
Fish meat type	BE	AMC	BE	AMC
Rainbow trout	0·32	0·32	0·42	0·39
Herring	0·74	0·88	1·87	1·84
Hake	0·59	0·63	2·19	2·40
Plaice	0·95	0·91	1·89	1·76

The rest of this paper illustrates work in the author's laboratories of the study of two alternative enzyme membrane electrode arrangements based on catalase and peroxidase, respectively, immobilised on nylon net with glutaraldehyde for the determination of hydrogen peroxide, and set in relation to analysis in various types of effluent and other environmental samples[24]. Also discussed are metal oxide membrane catalysts as alternatives to enzymes[25].

Finally, a possible rôle for enzyme electrodes in the determination of pollutants and other harmful materials by their inhibitory effects on enzyme action is briefly discussed[26].

The Peroxidase Electrode for Flow Injection Analysis

Here, the enzyme electrode, prepared from a nylon membrane with chemically immobilised peroxidase placed over platinum, is used in the mode as the working electrode in a three-electrode modified Stelte cell with hexacyanoferrate(II) as redox mediator in a phosphate buffer carrier stream[24]. As mentioned above, the electrode system may be operated at -100 mV vs silver–silver chloride, the low potential helping to offset the effect of redox-active interferents. Using a flow rate of $2 \cdot 0$ cm^3 . . min^{-1}, sample volume of $2 \cdot 0$ cm^3, a $0 \cdot 1$M phosphate buffer at pH 7, and $0 \cdot 01$M hexacyanoferrate(II), hydrogen peroxide standards (prepared by serial dilution of a $1 \cdot 75$M solution) injected in triplicate yielded a calibration line of $(r = 0 \cdot 999)$

$$\log \Delta I = 1 \cdot 06 \log ([H_2O_2]) - 2 \cdot 14 \qquad (3)$$

between $0 \cdot 0053$ and $1 \cdot 75$ nmol l^{-1} $(I, A; [H_2O_2], \text{mol l}^{-1})$. Calibration between $0 \cdot 0018$ and $1 \cdot 75$ mmol l^{-1} gave the line $(r = 0 \cdot 996)$

$$\log \Delta I = 1 \cdot 11 \log ([H_2O_2]) - 1 \cdot 98 . \qquad (4)$$

In each case[24], the slopes of greater than 1 are due to smaller than expected responses at low hydrogen peroxide concentration because of slow electrode response and/or peroxide decomposition.

Ten consecutive injections of $0 \cdot 018$ and $0 \cdot 18$ mmol l^{-1}, respectively, of hydrogen peroxide were reproducible to $<1\%$ coefficient of variation[24]. Also, in an assessment[24] of the effect of interferences by compounds likely to be present in industrial samples (e.g., sterilants used in food packaging and brewing vats) and effluents, only phenol, formaldehyde and ascorbic acid led to a reduction of more than 1% in signal for $17 \cdot 5$ mmol l^{-1} of interferent in $0 \cdot 175$ mM hydrogen peroxide but, of course, this can be as a result of chemical interference rather than electrochemical. Acetic acid, lactic acid, citric acid, glucose and sucrose were without effect, while ethanol

(0·1% v/v) led to a 4% enhancement of signal. In no case was there any permanent inhibition of signal.

In a study of the 1·75 mM hydrogen peroxide added to food type samples (yoghurt (1 g in 100 cm^3), milk, lemonade and lager beer), it was only orange juice which gave any significant drop in signal[24].

The electrode was stored at 4°C between measurements and had a lifetime of <5 months[24].

Dip-Type Catalase Electrode

A catalase enzyme electrode, suitable for use in alkaline waste streams, was fabricated by stretching a nylon net with chemically immobilised catalase over the PTFE membrane of a Yellow Springs Instruments (YSI) Model 5739 oxygen electrode[24]. This was calibrated in the dip mode by spiking hydrogen peroxide standards into a 0·1M Trizma buffer at pH 10, the resulting calibration line ($r = 0·988$) being

$$\log \Delta I = 0·984 \log([H_2O_2]) - 1·77 \qquad (5)$$

for 0·007 to 2·6mM hydrogen peroxide[24] (I, A; $[H_2O_2]$, mol l^{-1}). The slope of the electrode increased with age, demonstrating a lessening of the ability of the electrode to detect hydrogen peroxide at lower concentrations[24]. The lifetime was just 1 month for storage in 0·1M Trizma buffer at 4°C.

On reproducibility, three individual electrodes gave coefficients of variation of between 2·1 and 3·0% for 12 successive samples of 0·7 mM hydrogen peroxide, the mean signal for each of the three electrodes being 12·6, 11·1 and 10·4 μA, respectively[24].

Contact of the electrode with 17·5 mM sodium sulphide and cyanide effluent had devastating effects with complete elimination of response when the electrode was subsequently tested on 0·7 mM hydrogen peroxide[24]. Other interferents led to drops in signal, e.g., sodium hypochlorite (53%), sodium sulphite (11%), formaldehyde (4%), potassium thiocyanate (3%), and phenol-based effluent (2%). Phenol, potassium cyanate and sodium thiosulphate were without significant effect[24].

Metal Oxide Membrane Catalyst Electrodes

The use of immobilised metal catalyst membranes in association with an oxygen electrode for determining hydrogen peroxide was first proposed by Updike and co--workers[17] in a largely qualitative study. Schick and co-workers[18] used trapped lead(IV) oxide powder as a catalyst and obtained linear [hydrogen peroxide]/current calibrations between 0·001 and 1 mM hydrogen peroxide. The decomposition electrodes based on the oxide catalytic membranes can offer advantages, such as longer

shelf-lives, than is the case with enzyme systems. This has led to further studies of metal oxide membrane electrodes[25].

Metal oxide membranes were based on either membrane precipitation or powder entrapment[25]. During membrane precipitation, a dried acetate membrane was contacted concurrently on opposite sides with a solution containing the metal ion to be precipitated, and with sodium hydroxide (sodium iodide in the case of manganese(IV) oxide), respectively[25]. The membranes were then washed with deionised water, stretched over the Teflon membrane of a Clark oxygen electrode, and held in place by an 'O' ring. The powder-entrapped membranes were prepared by wetting the acetate membrane with saturated potassium chloride and placing it on a watch glass. The appropriate oxide powder (10 to 20 mg) was then placed on top, and the membrane fitted over the Teflon membrane of a Clark oxygen electrode[25].

Fifteen metal oxides were assessed for their utility in the catalytic type membranes, and manganese(IV) oxide, cobalt(III) oxide, lead(IV) oxide and ruthenium(III) oxide showed promise for further study[25]. All such membranes selected for more detailed study gave electrodes of rapid responses ranging from 20 to 45 s. The most sensitive was that based on manganese(IV) oxide, but although covering four orders of magnitude of hydrogen peroxide concentration (0·001 to 10 mmol l^{-1}), the calibration is non-linear[25]. The lead(IV) oxide membrane electrode, on the other hand, was linear in response between 0·0018 and 4 mmol l^{-1}. The cobalt(III) oxide membrane electrode sensed hydrogen peroxide down to 0·07 mmol l^{-1}, but the calibration showed a marked curvature[25]. The ruthenium(III) oxide membrane electrode gave a relatively poor calibration and seemed to be sensitive to the Trizma buffer[25]. As expected, the electrodes were best at high pH (7 to 11).

In a more detailed assessment, the manganese(IV) oxide membrane electrode showed good reproducibility, e.g., coefficient of variation of $<3\%$ for 12 consecutive readings[25]. Sodium sulphide and 17·5 mM sodium hypochlorite led to greatly reduced signals for 0·175 mM hydrogen peroxide, and a phenol-based effluent (1 400 mg l^{-1}) led to a $\approx 21\%$ reduction in signal. On the other hand, phenol, formaldehyde, sodium thiosulphate, sodium sulphate and potassium thiocyanate had little or no effect, and neither did a cyanide (500 mg l^{-1}) effluent[25].

When used for flow injection analysis in the cascade flow mode, the manganese(IV) oxide electrode, optimised for flow rate (2·00 cm^3 min^{-1}) and sample volume (1·37 cm^3) at pH 10 with respect to both 0·1M Trizma and phosphate buffers, gave good linearity ($r = 1·000$) for log–log plots up to 1·70 mmol l^{-1}, corresponding to (I, A; [H$_2$O$_2$], mol l^{-1})

$$\log \Delta I = 0·979 \log \left([H_2O_2]\right) - 2·63 \ . \tag{6}$$

The sample response reproducibility, assessed by alternate injections of 1·75 mM and 0·875 mM hydrogen peroxide at pH 10, gave a much improved coefficient of

variation $(\leq 1\%)$ over that quoted above for the corresponding dip-type electrode[25].

With regard to lifetime in the FIA mode, injection of 0·875 mM hydrogen peroxide in pH 10 Trizma buffer for 24 h led to a 20% drop in signal[25], attributed to a combination of catalyst inactivation and washout. Significant leaching and membrane discolouration was observed for electrode operation at pH 7.
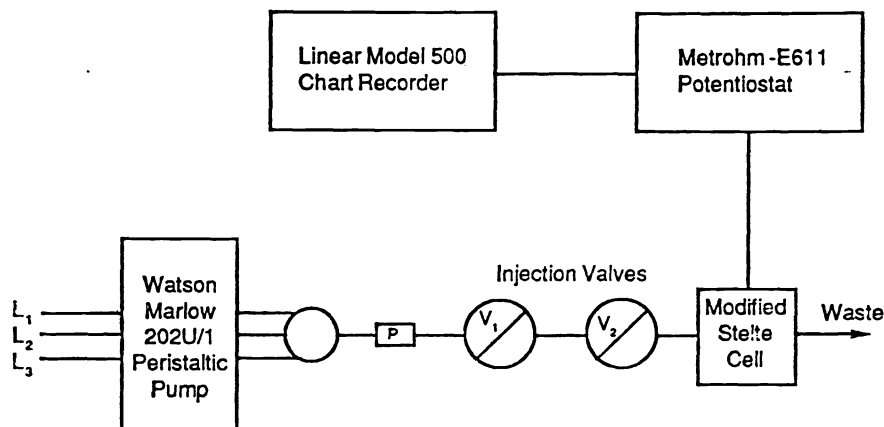

FIG. 1

Apparatus assembly for flow injection analysis type assessment of enzyme inhibition by heavy metal cations. Key: L_1 buffer; L_2 glucose in buffer; L_3 inhibitor, or inhibitor $+$ glucose in buffer; V_1 3 cm^3 injection valve, V_2 0·5 cm^3 injection valve; and P pulse suppressor (from ref.[26])
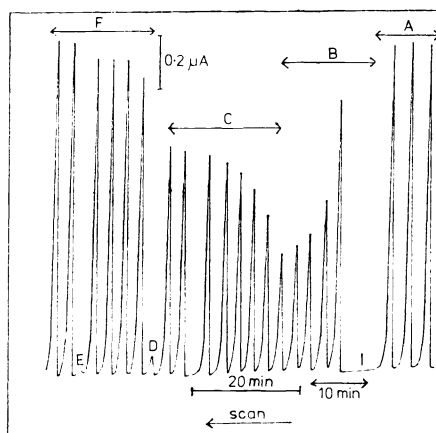

FIG. 2

Illustration of the inhibition by 1 mM copper(II) sulphate and regeneration of a glucose oxidase nylon mesh electrode. The 1 mM copper(II) sulphate in a background of carrier stream buffer was injected into the carrier stream for 10 min at I. Key of materials (0·5 cm^3) injected into buffer carrier stream: A 1 mM glucose; B 1 mM glucose into carrier stream treated with inhibitor; C 1 mM glucose; D and E 10 mM EDTA; and F 1 mM glucose (from ref.[26])

A MODEL FOR THE DETERMINATION OF ENZYME INHIBITOR TYPE POLLUTANTS AND SCOURGES

Many pollutants, such as heavy metals, and other scourges on society, such as narcotic drugs, are frequently enzyme inhibitors. Thus, the inhibitory effect of heavy metals on the catalytic action of glucose oxidase in the oxidation of glucose has been tested as a means of determining certain heavy metals from the reduction in the hydrogen peroxide produced in the enzyme catalysed reaction[26,27].

In principle, a FIA system for the analysis of glucose with a glucose oxidase electrode for sensing the hydrogen peroxide produced in the enzyme-catalyzed stage of glucose oxidation is fitted with an extra injection valve (Fig. 1) so that glucose and glucose–metal ion samples could be conveniently introduced[26]. The carrier stream arrangement of the system also permitted a choice of options (Fig. 1) such that glucose could be introduced through line L_2 (in addition to V_1 or with a metal ion through line L_3 (in addition to V_2). The unadulterated buffer carrier stream is conveyed along L_1.

The study showed that of the 16 metal cations studied, only copper(II), mercury(II) and silver(I) caused any significant inhibition[26]. Furthermore, the ability to reactivate the enzyme on the sensing electrode (Fig. 2) shows that, in principle, FIA lends itself to the determination of enzyme inhibitors.

The tangible effect of the enzyme inhibition by the metal cations is a loss of slope for the calibrations of current response vs glucose substrate concentration. Enzyme regeneration (with EDTA to strip inhibitor from the enzyme), although less easy with mercury(II), and difficult with silver(I), was sufficiently good for copper(II) for a calibration ($r = 0.994$) to be obtained between $2.5 \cdot 10^{-4}$ M and $5 \cdot 10^{-3}$ M Cu^{2+} according to the literature[20] (I, A; $[Cu^{2+}]$, mol l^{-1})

$$\Delta I = -9.49 \cdot 10^{-7} \log\left([Cu^{2+}]\right) + 4.84 \cdot 10^{-8} . \tag{7}$$

CONCLUSION

Several electrode systems can be used for analyses of environmental interest. Among these, ISEs in potentiometry and amperometric electrodes with catalytic membranes are of particular interest. With enzyme electrodes, the use of mediated systems and of catalytic decomposition of hydrogen peroxide offer useful alternatives for circumventing certain interferences.

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