Fiber-optic Biosensor Based on Immobilized Lipase and Its Analytical Applications

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The construction and characterization of a lipase-based fiber-optic biosensor are described and its application in the analyses of triglycerides and pesticides is evaluated. The sensing phase of the biosensor consist of lipase covalently immobilized on isothiocyanate-activated glass beads mixed with thymol blue bound on modified glass beads, then packed at the tip of an optical fiber bundle. Characterization studies showed that the sensor response was affected by pH, buffer concentration, buffer type, temperature, enzyme loading, and the presence of NaCl and Triton X-100). Under optimal conditions, high reproducibility in the sensor response can be achieved. The sensor proved sensitive for the determination of triacetin and triolein, in the following respective dynamic ranges: 10-250 mM and 5-80 mM. However, the biosensor showed less applicability for the detection of carbamate or organophosphate pesticides. Measures for the improvement of the sensor response are also proposed.

Key words: fiber-optic biosensor, immobilized lipase, triglyceride biosensor, pesticide biosensor

INTRODUCTION

The determination of triglycerides (neutral lipids) is of clinical and industrial importance. The analysis of triglycerides in blood serum is one of the many tests commonly performed in the diagnosis and/or treatment of lipoprotein disorders [1]. Triglyceride measurements are also routinely carried out in the food, fat, and oil industries for assessing fat levels in food or oil samples [2-3]. These analyses are traditionally performed using chemical methods involving organic extraction, alkaline hydrolysis, chromogenic reactions, etc. Liquid and gas chromatographic techniques are also commonly used for triglyceride quantitation [4-6]. Enzyme-based procedures have also been increasingly utilised in the assay of triglycerides [7]. In these enzymatic methods, the lipid molecule is normally measured through coupled-enzyme reactions directed on the glycerol component of the triglyceride [8-11]. The release of the glycerol is first effected by a preliminary chemical or lipase-catalysed hydrolysis of the triglyceride. Afterwards, glycerol is assayed through any of various coupled-enzyme approaches (see Table 1), where the measured end product is related back to the amount of glycerol and, in turn, to the triglyceride content.

A few enzyme electrodes for the determination of triglycerides have also been realized. Satoh *et al* [12] described a thermistor device for measuring the heat of protonation produced when a triglyceride is passed through a column, which contained triacylglycerol lipase covalently-bound to controlled-pore glass. In a different set-up, a flow-through pH electrode was used to detect the fatty acids liberated from the triglycerides, which has been acted upon by lipase covalently bound to polystyrene sheets in a reactor column [13]. Nakako *et al.* [14] reported the use of ion-sensitive field effect transistors, with immobilised lipase at its sensitive area, as a sensor for neutral lipids.

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Table 1. Some coupled-enzyme reactions utilised for the analysis of triglycerides based on the measurement of liberated glycerol: ATP = adenosine triphosphate, GK = glycerol kinase, ADP = adenosine diphosphate, GPD = glycerol-3-phosphate dehydrogenase, PK = pyruvate kinase, LDH = lactate dehydrogenase, GPO = glycerol-3-phosphate oxidase, HRP = horseradish peroxidase, GDH = glycerol dehydrogenase, NAD(H) = oxidised (reduced) nicotinamide adenine dinucleotide.

Enzyme Reaction	Ref
glycerol + ATP $\xrightarrow{GK}$ glycerol-3-phosphate + ADP glycerol-3-phosphate + NAD$^+$ $\xrightarrow{GPD}$ dihydroxyacetone phosphate + NADH + H	8
glycerol + ATP $\xrightarrow{GK}$ glycerol-3-phosphate + ADP ADP + phosphoenolpyruvate $\xrightarrow{PK}$ ATP + pyruvate pyruvate + NADH + H$^+$ $\xrightarrow{LDH}$ lactate + NAD$^+$	9
glycerol + ATP $\xrightarrow{GK}$ glycerol-3-phosphate + ADP glycerol-3-phosphate + O$_2$ $\xrightarrow{GPO}$ dihydroxyacetone phosphate + H$_2$O$_2$ H$_2$O$_2$ + p-hydroxyphenylacetic acid $\xrightarrow{HRP}$ fluorescent product	10
glycerol + NAD$^+$ $\xrightarrow{GDH}$ dihydroxyacetone + NADH + H$^+$	11

In this paper, a fiber-optic lipase-based biosensor for triglycerides is described. The reagent phase of the optrode consists of a mixture of controlled pore glass-bound lipase and pH-sensitive glass beads, which are packed at the tip of a fibre-optic probe. The response characteristics of the sensor were investigated and optimised. The sensor was tested for the assay of three triglycerides, namely triacetin, tributyrin, and triolein. Its application to the assay of organophosphate and carbamate pesticides was also attempted.

EXPERIMENTAL

Materials. All reagents were obtained from commercial suppliers and were utilized as received. Tris(hydroxymethyl)-aminomethane hydrochloride (Tris), KH$_2$PO$_4$, Triton X-100, tributyrin (glyceryl tributyrate), and diethyl 4-nitrophenyl phosphate, tech., 90% (paraoxon) were bought from Aldrich Chem. Co. (Gillingham, Dorset, UK). Lipase from *Candida cylindracea* (L8525, 300000 units/mg protein), triacetin, triolein (99%), and isothiocyanate glass (Sigma G4893, average pore size 500 Å, 200-400 mesh, 39 μmol isothiocyanate/g glass) were purchased from Sigma Chem. Co. Ltd. (Poole, Dorset, UK). The carbamate pesticides, carbaryl and carbofuran, were obtained from Riedel-de Haën (Seelze, Germany). All other reagents used were of analytical reagent grade.

Apparatus. Fiber-optic measurements were carried out using the instrumentation system depicted in Fig. 1. The set-up consists of a xenon lamp (12V, 50W), whose radiation
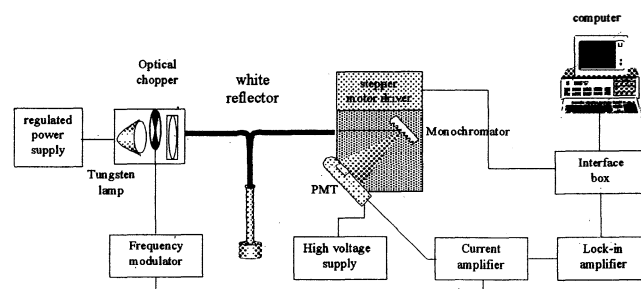

Fig. 1. Schematic diagram of the fiber-optic instrumentation.

was modulated by an optical chopper (Bentham 218) and focused into the end of one arm of a bifurcated optical fiber. The 16-polymer-fiber bundle arm directs the radiation to the common tip of the bifurcated fibre, where it interacts with the reagent phase and is reflected back to the other 16 collecting optical cables. The reflected radiation is then carried into a grating monochromator (ISA Instruments, Yobin-Ivon Type H10-61) and detected by a photomultiplier tube (Hamamatsu R446). The photodetector signal was enhanced using a current amplifier (Bentham 286) and a lock-in amplifier (Bentham 223). The amplifier output was displayed on a digital voltmeter (Bentham 217) and recorded by a microcomputer (Viglen II, PC-AT) through a 12-bit A/D interface system (General Purpose Interface System, 3D Digital Design and Development Ltd.).

Procedure

Immobilization of lipase. Lipase was immobilized through a direct covalent coupling method on preactivated isothiocyanate glass. Each batch of the immobilized enzyme preparation was made by adding 200 µl of lipase solution, (prepared by dissolving 2.5 mg of the enzyme in 250 µl of pH 8.5, 0.05M borate buffer), to a 5 mg sample of the isothiocyanate glass in a small glass vial (1 cm dia. x 5 cm ht.). The mixture was gently swirled then kept in the refrigerator for at least 2 h to allow the covalent binding. Afterwards, the excess enzyme solution was decanted and the glass material was repeatedly washed, first with cold distilled water, then with pH 7.0, 0.1M phosphate buffer.

Sensor construction and flow-cell configuration. To prepare the sensitive reagent phase of the lipase-based optical sensor, the 5 mg portion of the isothiocyanate glass with bound enzyme was mixed with an equivalent amount of a thymol blue-modified aminopropyl glass beads. The immobilization of thymol blue on the glass beads follows the procedure described elsewhere [15] except that a 5 mg portion of support matrix is used in the present study.
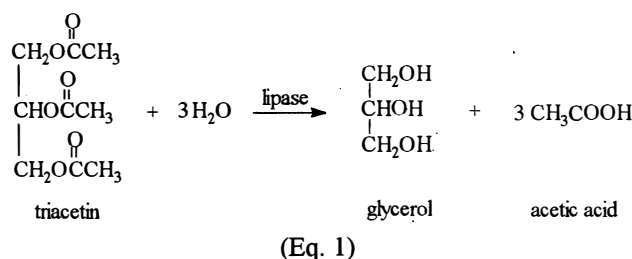
The optical biosensors were constructed by pipetting small amounts of the immobilized reagent into the microwell (100-150 µm deep x 1.5 mm dia.) of a bifurcated fibre-optic sensor head (see inset of Fig. 2). These were then blotted dry using an absorbent paper and carefully packed until an even and compact solid layer fills the microwell at the fibre-optic tip. The solid particles were then retained using a fine nylon mesh, which in turn is supported in place using a plastic support tube that snugly fitted the sensor head. The fibre-optic probe is then inserted into a flow cell assembly, which was machined from a Perspex cylinder (1 cm. dia x 1 cm. height). The flow cell device incorporated two 19G (1mm o.d., 20 mm length) stainless steel needles, positioned dia-

metrically opposite each other, to serve as solution inlet/outlet. The bottom end of the flow cell was also fitted with a white PTFE plug (at 1.5 mm distance from the probe tip) to provide a reflective surface backing. The internal volume of the flow cell was approximately 25 µl.

RESULTS AND DISCUSSION

Characterization of response of the triglyceride sensor. The effect of various experimental parameters on the response of the lipase-based fibre-optic biosensor was investigated, with the view of optimizing the conditions for the measurement procedure. The variables studied include pH, buffer composition, buffer concentration, ionic strength, temperature, presence of added detergent (Triton X-100), and enzyme concentration.

For the characterization studies, triacetylglycerol (triacetin) was used as the test substrate solution. This substrate is hydrolyzed by lipase, according to the reaction shown below:

$$CH_2OCCH_3 \mid CHOCCH_3 + 3H_2O \xrightarrow{\text{lipase}} CHOH + 3 CH_3COOH \mid CH_2OCCH_3$$

triacetin   glycerol   acetic acid

(Eq. 1)

This reaction results in a decrease in pH around the sensing region, thus leads to an increase in the measured signal for the immobilised thymol blue. An example of the optrode signal tracing during the injection of triacetin is shown in Fig. 3.
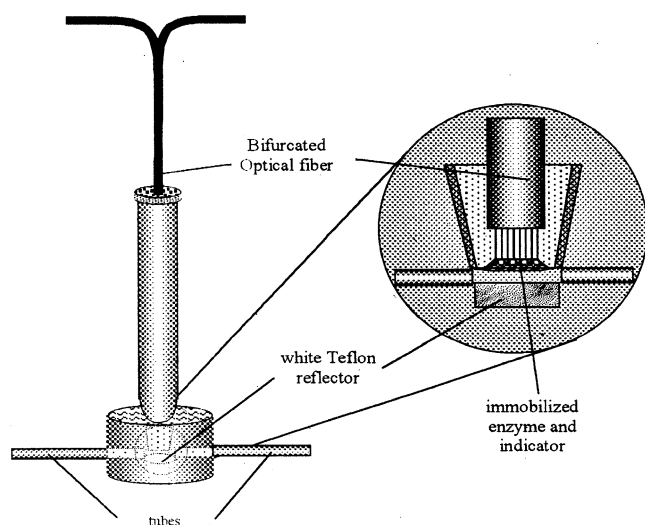

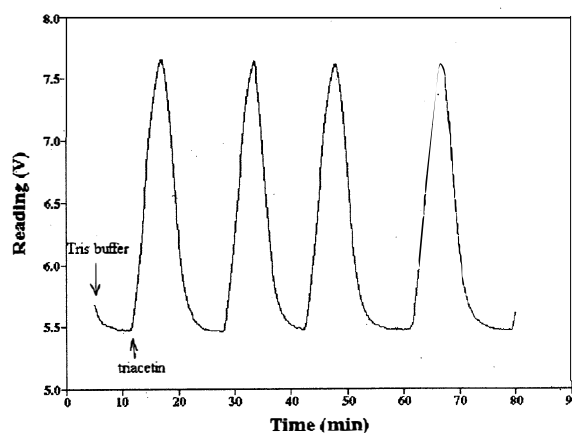Fig. 2. Construction of the fiber-optic sensing head.

Fig. 3. Response curves of lipase optode on repetitive exposure to triacetin (250 mM). Experimental conditions: carrier solution = pH 7.5, 7.5 mM Tris buffer, temperature = 25°C, flow rate = 0.5 ml min⁻¹.

Effect of pH and chemical nature of the buffer. The response of the lipase-based optode was determined under different pH conditions using two buffer systems (Tris buffer and phosphate buffer). Buffer solutions of defined pH values were made by titration of the stock 7.5 mM phosphate and Tris solutions with 2M NaOH to the appropriate pH values. The buffered triacetin solutions (50 mM concentration) were then prepared by dilution of the stock 250 mM aq. solution with the respective buffer solutions. The lipase optrode responses under these different pH conditions are shown in Fig .4. The profiles of the pH-dependence of the sensor response in the two mentioned buffer solutions are similar. The smallest signal changes are recorded at the pH values, which are nearest to the pK_a of the buffer systems (phosphate ~ 7.2 and Tris ~ 8.1). This behaviour follows the pH response pattern normally observed with sensors based on the detection of pH changes. For this work, Tris buffer, specifically adjusted to pH 7.5, and was deemed suitable for carrying out further studies and sensor measurements. With this buffer, large signals are obtained for the sensor, and at this pH, sufficient buffering capacity is still maintained to yield stable baseline readings and reasonable baseline recovery times (typically 7-11 min).

Effect of buffer concentration. Having chosen Tris as the working buffer solution, the sensor response was then measured at different concentrations of the Tris buffer, all adjusted to pH 7.5. For these measurements, 50 mM triacetin solutions, made up in the respective Tris buffer solutions, were again utilized as the test substrate. As expected, the sensor sensitivity progressively decreased with an increase in the buffer concentration (Fig. 5). This is a consequence of the greater extent of neutralization of the enzymatically generated acetate species with an increasing amount of the buffer reagent. A 10 mM buffer concentration was chosen as a suitable solution concentration for carrying out further enzyme reactions. This is the concentration where the optimum response-to-baseline recovery time ratio was attained.

Effect of temperature. The effect of temperature variations on the substrate response of the lipase-based sensor was also studied. The temperature changes were noted to have a slight effect on the lipase-based optode (Fig. 6). As temperature was increased (between 15 to 50°C), the magnitude of the biosensor response also gradually decreased, which can be ascribed to the slow denaturation of the immobilised enzyme. For convenience, 25°C was chosen as the working temperature for subsequent measurements, considering that enzyme activity remains high at this temperature.

Effect of NaCl concentration. The effect of the variations in NaCl content of the reaction medium on the response of biosensor was also investigated. For this purpose, buffer so-
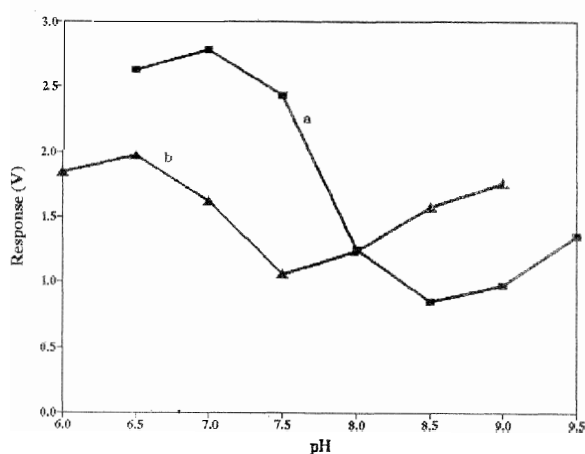

Fig. 4. Effect of pH on the response of lipase optrode to 50 mM triacetin: a.) 7.5 mM Tris buffer, b.) phosphate buffer. Experimental conditions: temperature = 25°C, flow rate = 0.5 ml min⁻¹.
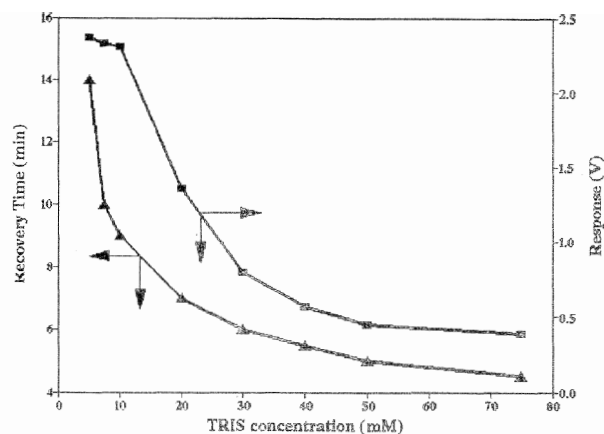

Fig. 5. Effect of Tris buffer concentration on the response and recovery time of lipase optrode to 50 mM triacetin exposure. Experimental conditions: temperature = 25 °C, flow rate = 0.5 ml min⁻¹.
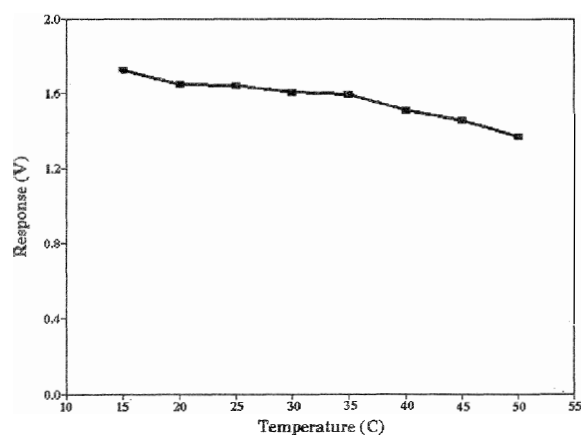

Fig. 6. Effect of temperature on the response of lipase-based optrode to 50 mM triacetin. Experimental conditions: carrier solution = pH 7.5, 7.5 mM Tris buffer, flow rate=0.5 mL min⁻¹
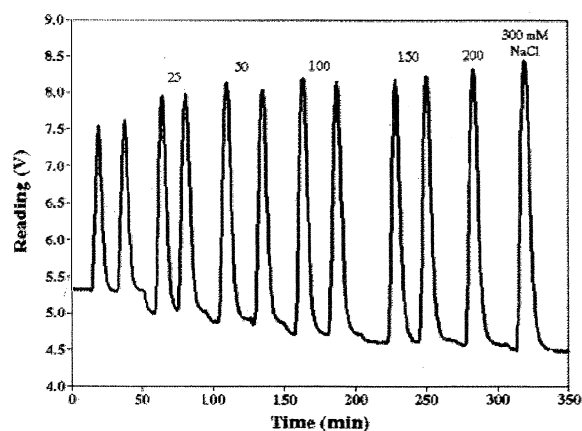
Fig. 7. Time response curve for the lipase-based optrode under buffer and substrate solutions of varying NaCl concentration. Experimental conditions: carrier solution=pH 7.5, 7.5 mM Tris buffer, flow rate= 0.5 ml min⁻¹, triacetin = 50 mM.
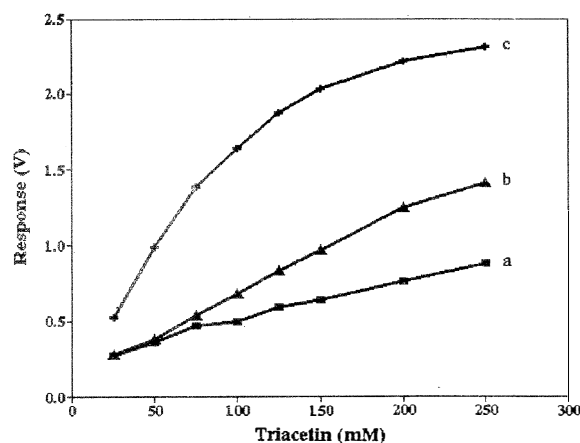

Fig. 8. Effect of enzyme loading on the response of lipase-based optrode to triacetin; graph labels correspond to the reagent phases described in Table 2. Experimental conditions: carrier solution = pH 7.5, 10 mM Tris buffer, temperature = 25°C, flow rate= 0.5 ml min⁻¹.

lutions (pH 7.5. 7.5 mM Tris) with varying amounts of NaCl were prepared and were also used for making up the respective substrate solutions (50 mM triacetin). Figure 7 reveals that at higher NaCl concentration, the substrate response of the biosensor was also greater. The increase in response was the result of the composite decrease in the baseline level and increase in the response peak heights as the NaCl concentration was increased. The enhancement in the response though can be seen to be more dramatic only between 0 and 100 mM NaCl. At higher NaCl concentration, the improvement in the sensor signal becomes less significant. The effect of NaCl on the sensor behaviour can be ascribed to the combined effects of the activation of lipase, the enhanced ionization of the enzymatically released products, hence yielding higher signals and the modification of the response of the optical pH transducer. For this sensor, a 0.1M NaCl concentration appears a reasonable buffer ionic content for carrying out subsequent analysis. The sensor sensitivity in this concentration was acceptable enough.

Effect of enzyme loading. Reagent phases with varying enzyme loadings were also prepared and tested for their responses to substrate exposures. The preparation of these different enzyme reagent phases follows the procedure given in the Experimental section. For the present case, however, the amount of lipase solution (4.0 mg lipase/400 μl borate buffer) added to the isothiocyanate glass was varied by dilution with borate buffer according to the proportions given in Table 2.

The calibration curves obtained for these three enzyme reagent preparations are shown in Fig. 8. It is clear that the sensor sensitivity and dynamic range are both a function of the enzyme loading. Small amounts of enzyme result in a wider dynamic range but the sensor sensitivity was lower.

Table 2. Preparation of immobilized lipase with varying enzyme loadings.

Reagent	Vol. Lipase Solution (μl)	Vol. Borate Buffer (μl)
a	50	150
b	100	100
c	200	0

Higher enzyme loadings yielded more sensitive measurements, however the calibration curve obtained also had narrower linear region.

Effect of added detergent (Triton X-100). In order to test the effect of added detergent on the response of the lipase-based optrode, buffer solutions (pH 7.5, 10 mM Tris/0.1M NaCl) containing varying amounts (0%, 0.05%, 0.10%, 0.50%, and 1.0% m/v) of Triton X-100 (polyethylene glycol tert-octylphenyl ether) were prepared and also used for the preparation of the test substrate solutions (50 mM triacetin). As can be observed in Fig. 9 the sensor response varied with the amount of added detergent. The highest response was obtained at 0.05% m/v level of Triton X-100. Although within the studied detergent concentration range, the change in sensor signals was consistently higher for substrate solutions with detergent as compared with the unmodified substrate solution. This behaviour can be attributed to the enhancement of the solubility of triacetin in the detergent-containing buffer solutions, thereby leading to the increase in the effective concentrations of the analyte solutions, hence the increase in response. The signal increase may also be ascribed to the possible activation effect of the detergent on the enzyme and/or the improvement in the substrate-enzyme interaction when in the presence of the detergent [16].
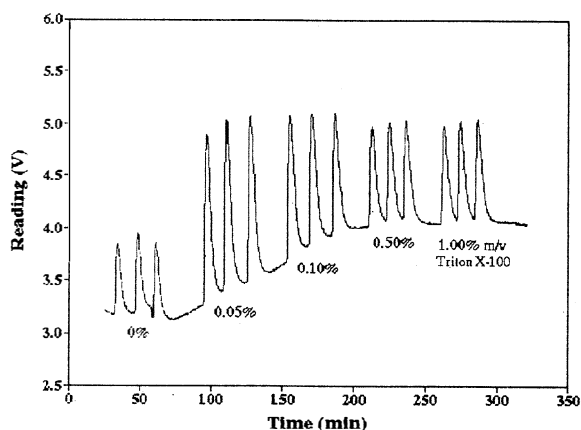
Fig. 9. Response cure of lipase-based optrode showing the effect of added Triton X-100. Experimental conditions: carrier solution = pH 7.5, 10 mM Tris buffer, temperature = 25°C, flow rate = 0.5 ml min⁻¹, triacetin concentration = 50 mM.

Fig. 11. Response of the lipase-based optrode to triacetin and tributyrin solutions. Experimental conditions: carrier solution = pH 7.5, 10 mM Tris buffer, temperature = 25°C, flow rate = 0.5 ml min⁻¹, NaCl concentration = 0.1 M.
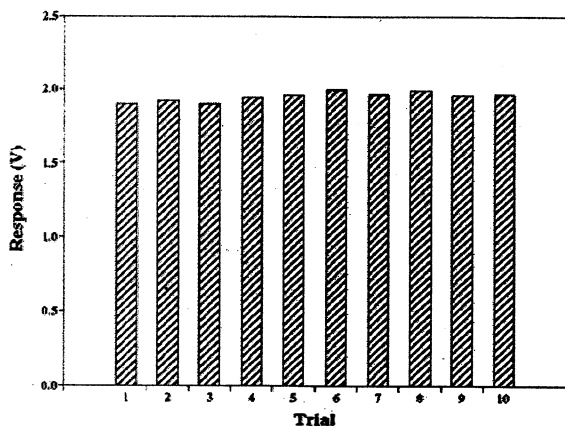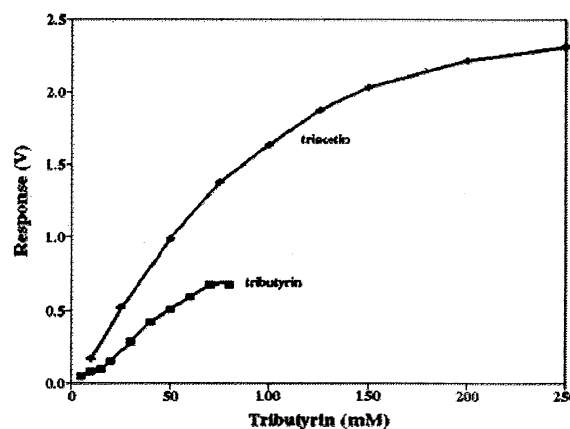

Fig. 10. Reproducibility of response of lipase-based optrode to repetitive exposure to 50 mM triacetin. Experimental conditions: carrier solution = pH 7.5, 10 mM Tris buffer, temperature = 25°C, flow rate = 0.5 ml min⁻¹, NaCl concentration = 0.1 M.

Another notable effect of Triton-X100 on the sensor response was the modification of the baseline signal. The drift in baseline level actually accounted for the main variations in the peak heights of the response as the concentration of the detergent was changed. Significant baseline drift occurred especially during the transitions between the pure buffer solution and the buffer-0.10% Triton X-100 mixture. The change in the background signal was not due to the variations in the pH between the different buffer solutions, since the pH of the solutions was verified to differ only by around ±0.01 pH units from each other. The increase in the baseline level is more likely the result of the modification in the environment of the immobilized indicator resulting from the adsorption of Triton X-100 entities on the solid support. It

has already been reported [17] that co-adsorbed detergents can influence the pH behaviour of a physically immobilized indicator.

Reproducibility of response. Figure 10 shows the fibre-optic sensor response on repetitive exposure to 250 mM triacetin solution. High reproducibility (r.s.d. = 1.69% for n = 11) was obtained for these measurements. However, slightly lower precision (r.s.d. = 4.95% for n = 8) was achieved when the buffer and substrate solutions were added with Triton X-100. This was due to the shifting baseline level and the signal interference from bubbles (foams) that are readily formed in these solutions. Loose reagent packing, entrapped bubbles, and insufficient washing of the enzyme reagent phase, are also the major elements noted to impair the precision of the lipase-based optrode.

Calibration curves for triglycerides. Figure 11 compares the response of the optical lipase-based biosensor to various concentrations of triacetin and tributyrin. For the triacetin calibration curve, the triacetin standard solutions were prepared by direct dissolution of the analyte with Tris buffer, and with no further addition of the detergent. Considering the complications arising in the presence of Triton X-100 (bubbling, long sensor preconditioning time, and baseline drift) and the high natural solubility of triacetin in the buffer solution, it was thought that the addition of Triton X-100 was no longer necessary. However, for tributyrin, the inclusion of Triton X-100 was required in order to prepare a clear solution (or at least a stable emulsion) of the substrate. With a 5 % m/v Triton X -100 content, clear substrate solutions were produced up to 50 mM concentration. At higher analyte concentrations, the substrate preparations became more cloudy or whitish. It was noted though that the sample turbidity did not significantly affect the response of the optrode.
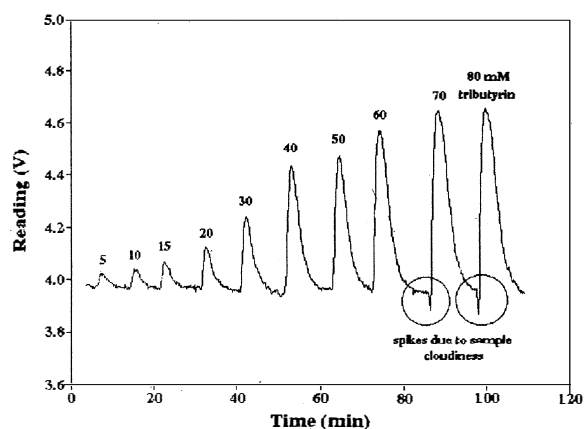
Fig. 12. Time response curve of the lipase-based optrode for tributyrin. Experimental conditions: carrier solution = pH 7.5, 10 mM Tris buffer, temperature = 25°C, flow rate = 0.5 mL min⁻¹, NaCl concentration=0.1M, Triton X-100 concentration = 5% m/v. Encircled area illustrates the spikes produced on exposure of the sensor to cloudy substrate solutions.

The introduction of cloudy substrate solutions into the sensor manifested only as small negative spikes in the time response curve of the sensor (see Fig. 12).

The response of the lipase optrode to the two triglycerides closely follows the solubility characteristics of the analyte species and the ionization properties of the respective organic acid components (acetic acid and butyric acid) of the substrates. Higher signals were obtained for triacetin due to its greater water solubility than tributyrin. Furthermore, larger pH changes resulted from the release of acetic acid than butyric acid because of the slightly higher ionisation constant of the former (HOAc:1.754×10^{-5} and HOBu: 1.515×10^{-5} at 25 °C [18]). The dynamic ranges for triacetin and tributyrin are 10-250 mM and 5-80 mM, respectively. The linear region for both substrates appear to be similar though (10 to 75 mM).

The response of the sensor to emulsified triolein solutions (containing 10% m/v Triton X-100) was also assessed. The sensor showed no sensitivity to this substrate however. A stopped-flow procedure was also implemented (stopped-flow time = 5 min) but there was no detectable improvement in the sensor response. The absence of response may be due to the slow enzyme reaction with triolein, the weak acidity at low levels of the liberated oleic acid and the low water solubility of both triolein and oleic acid.

Application of lipase-based optrode to pesticide detection.
The feasibility of using the lipase-based optrode for the detection of carbamate or organophosphate pesticides was also tested. Some reports in the literature [19-21] have already

indicated that lipase can be useful for the assay or detection of pesticides, by measuring the inhibitory effects of these compounds on its activity.

Following on the above literature reports, it appears logical to also extend the application of the already presented lipase-based optical biosensor for the detection of organophosphate or carbamate pesticides. For this purpose, triacetin was also selected as the substrate solution for assaying the enzyme activity in the fibre-optic sensor. Although, it is possible to employ other substrates, which yield fluorescent or coloured products, the presently available substrates are not wholly suitable for such direct optical transduction of the enzyme activity. For example, measurements with 4-methylumbelliferone heptanoate as the substrate can only be made with the use of more expensive glass or quartz optical fibres due to the lower excitation/emission wavelengths (λ_{ex} = 330 nm and λ_{em} = 450 nm) [21] for this reagent. A different fluorimetric substrate, dibutyrylfluorescein, can be detected at higher wavelengths, but suffers from a small Stokes' shift. Furthermore, it undergoes spontaneous hydrolysis and has a lower water solubility [20]. Other reagents, e.g. Orange I laureate [22], have also been utilised as lipase substrates but they are not readily commercially available and entail laborious synthetic preparations. The use of triacetin as the indicating substrate reagent can confer several other advantages though. It is hydrolyzed rapidly by lipase that the reaction can be carried out at ambient temperature and without or with only minimum incubation. Furthermore, this substrate has a high degree of water solubility thus lends to the easy preparation of the reagent solution and prevents any consequent complication arising from the use of organic solvents or emulsified substrates.

Inhibition measurements were performed in a single line, dual valve flow-injection manifold. The first valve was used to inject the substrate solution (250 mM triacetin) for assaying the enzyme activity and the second valve was for introducing the pesticide samples. The test pesticides solutions for these measurements were prepared from 1 mM acetonoic stock pesticide solutions, which were then diluted with the Tris buffer solution to the desired concentration. However, tests with 1×10^{-5} M concentrations of aldicarb, carbofuran, and paraoxon revealed that the lipase optrode was not really sensitive to the inhibition by these pesticide samples. Similar results were obtained even with 1×10^{-4} M samples or when using a reagent preparation with lower enzyme loading (reagent phase B from Fig. 8). It is obvious from the result that the present lipase-based optrode is not suitable as a pesticide sensor, as opposed to the data suggested in the literature [19-21]. The insensitivity of the optrode to pesticide may be the result of a stabilized conformation of the lipase, particularly enhanced by the immobilization of the enzyme on the glass support. In its immobilized state, the essential serine moiety in the active site of lipase may have been favourably concealed from the possible attack of the

phosphorylating or carbamylating pesticides. Consequently, enzyme inhibition is averted and pesticide detection was not possible. No further studies were carried out on improving the perticide sensitivity of the lipase-based optrode.

CONCLUSION

The construction and characterization of a lipase-based fiber-optic biosensor have been described and its application in the analyses of triglycerides and pesticides (organophosphates and carbamates) was evaluated. Characterization studies showed that the sensor response was affected by pH, buffer concentration, buffer type, temperature, and enzyme loading. Externally added NaCl and detergent (Triton X-100) also have significant influences on the sensor behaviour. The enzyme loading of the sensor can be varied and used to control the sensitivity and its dynamic range.

When tested for its applicability in the determination of three test triglycerides (triacetin, tributyrin, and triolein), however, the optical biosensor was observed to be only sensitive to triacetin and tributyrin. The dynamic ranges being 10 - 250 mM and 5 - 80 mM for triacetin and tributyrin, respectively. The linear ranges for these substrates though was almost similar (10 - 75 mM). The sensitivity pattern for the different triglycerides appear to closely follow their water solubility, the acidic strength of their respective organic acid component, and the water solubility of these acid products. Sensitive response to higher triglycerides may be achieved by using other emulsifying agents to improve their solubility, increasing the enzyme loading of the enzyme optrode, allowing a longer enzyme-substrate contact time, and utilising a more sensitive optical pH transducing reagent or devising an optical transducer for glycerol.

The lipase-based optrode was also tested for its applicability in the detection of carbamate and organophosphate pesticides. However, the results were not promising even when using a higher concentration (100 µM) for these pesticides. The pesticide insensitivity may be ascribed to the possible stabilized conformation of the immobilised lipase and sterically hindered pesticide binding sites in the enzyme. The use of other types of enzyme supports may improve this sensitivity.

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