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Silica Sol-Gel Immobilized Amperometric Biosensor For Hydrogen Peroxide[#]

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Abstract

An amperometric enzyme electrode for hydrogen peroxide was developed via an easy and effective enzyme immobilization using the sol-gel technique. The enzyme electrode comprises horseradish peroxidase (HRP) immobilized by the silica sol-gel matrix on a carbon paste electrode. Hydrogen peroxide was detected in the presence of ferrocyanide as a mediator to transfer electrons between the electrode surface and hydrogen peroxide. The effect of operational parameters, such as operating potential of the working electrode, mediator concentration, pH, and the thermal stability, were explored for optimum analytical performance by using amperometric method. A linear calibration curve for hydrogen peroxide measurement was obtained in the range of $2x10^{-5}$ to $2.6x10^{-3}$ M under the optimized conditions. The apparent Michaelis-Menten constant (K_m^{app}) of the enzyme electrode is 4.8 mM. The performance of this enzyme electrode was also investigated using flow-injection analysis (FIA). The enzyme electrode retained about 60% of its activity after 35 days of storage in a phosphate buffer at 4C°.

Keywords: sol-gel technique, peroxide, horseradish peroxidase, amperometry, FIA, ferrocyanide

1. Introduction

The determination of hydrogen peroxide is of practical importance in chemical, biological, clinical, and many other fields. Extensive techniques have been developed for this purpose, e.g. titrimetry [1], spectrometry [2], chemiluminescence [3] and electrochemical methods [4-10]. Among the electrochemical methods, the amperometric biosensors for hydrogen peroxide based on electron transfer between an electrode and immobilized horseradish peroxidase (HRP), so catalyzing the reduction of hydrogen peroxide, are promising for the fabrication of selective and sensitive hydrogen peroxide sensors [5-8].

Enzyme electrodes have been studied widely for the two decades and many research efforts have been devoted to them. A very important factor in enzyme-based electrodes development is the immobilization technology of enzymes. The common conventional methods of immobilization involve non-covalent (entrapment and adsorption) or covalent schemes [11]. Each of these immobilization procedures has its own advantages and disadvantages, and the procedures can have a significant consequence in terms of cost and labour. Therefore, it should be obvious that biosensor development is limited somewhat by the lack of a simple and generic enzyme immobilization protocol. As a result, a simple and reliable method to immobilize and stabilize active enzymes so that they can be located at an electrode would offer many advantages.

Recently, sol-gel methods have provided an alternative way to immobilize enzymes. The enzymes in the sol-gel matrix have been reported to retain their functional characteristics to a large extent [12-14]. Sol-gel process is a low-temperature technology for the production of ceramic materials by hydrolysis and polycondensation of alkoxides. In 1990, Braun and co-workers first demonstrated the possibility of protein immobilization in a sol-gel silica matrix [15]. This class of bioceramic materials was further applied to

produce silica based photometric biosensors [16]. The porous sol-gel inorganic matrix is particularly attractive for electrochemical biosensors development since the matrix possesses physical rigidity, chemical inertness, high photochemical, biodegradational and thermal stability, and experiences negligible swelling in aqueous solutions. Several papers on the immobilization of glucose oxidase within a sol-gel matrix for the development of glucose biosensor have been reported [17-22]. Recently we developed a mediated amperometric glucose sensor modified by sol-gel method in a 'sandwich' configuration demonstrated improved sensitivity and stability [23].

In this paper, we report the first attempt to immobilize HRP in a thin silica sol-gel matrix derived from tetramethoxysilane (TMOS) on a carbon paste electrode. The determination of hydrogen peroxide was performed in the presence of a hydrogen donor, ferrocyanide in a phosphate buffer solution. Hydrogen peroxide oxidizes ferrocyanide to ferricyanide in the presence of HRP, and ferricyanide which consequently can be reduced at the hydrogen peroxide biosensor at a potential of -100 mV vs. Ag/AgCl reference electrode. The optimized conditions for the fabrication and analytical performance of the enzyme electrode were studied. Possible interferences and the stability of the electrode were evaluated. The determination of hydrogen peroxide by using the silica sol-gel immobilized HRP enzyme electrode was also performed under FIA system.

2. Experimental

2.1. Reagents

Tetramethoxysilane (TMOS, 99%) was obtained from Janssen Chimica. Horseradish peroxidase (HRP, EC 1.11.1.7, 727U mg^{-1}) and hydrogen peroxide (30%) were obtained from Fluka. Cetyltrimethylammonium bromide (CTAB) was obtained from Aldrich and prepared as 3.8% (w/v) solution in methanol. All other chemicals were

analytical grade and used as received without further purification. All aqueous solutions were prepared in distilled deionised water.

2.2. Procedures

Preparation of a typical TMOS stock sol-gel solution

A homogeneous stock sol-gel solution was prepared within 5 minutes by vigorously mixing 600 µl of methanol, 50 µl of TMOS, 10 µl of 3.8% CTAB solution, 10 µl of 5 mM NaOH and 60 µl of H_2O in a small test tube at room temperature. This stock sol-gel solution was then cooled to 4°C immediately after mixing. The surface active agent, CTAB, was added to the stock sol-gel solution to prevent the sol-gel film on the electrode from fracture [16]. The catalyst selected was 5 mM NaOH so that the solution pH was not too extreme to denature the enzyme and an entire sol-gel film could be formed within a reasonable time. This stock solution was freshly prepared daily just before the fabrication of the enzyme electrode. This formulation was changed accordingly when certain experimental parameters were investigated.

Construction of a carbon paste electrode (CPE)

A CPE was used as the basic electrode for the sol-gel modified peroxide electrode. It was prepared as follows: 5 g of graphite powder (Merck fine power extra pure) and 3 ml of silicon oil were mixed thoroughly in a mortar to form a homogenous carbon paste. A portion of the carbon paste was packed into one end of a rigid plastic tubing (4.5 mm i.d., 6.5 mm o.d.). A copper wire was inserted through the opposite end to establish electrical contact. The CPE surface was smoothed on a piece of paper.

Construction of the silica sol-gel immobilized HRP enzyme electrode

Initially, 5 μl of HRP solution (5 mg HRP was dissolved in 200 μl of phosphate buffer (pH 7.0) solution containing 5 μl glycerol) was pipetted onto the surface of CPE, and distributed gently over the entire surface of the CPE with the aid of a sealed melting point capillary tube. This was to ensure a complete coating of the CPE by HRP enzyme solution. The presence of a small amount of glycerol in the enzyme stock solution acts as an emollient. Thus, HRP could spread easily on CPE and after drying, it did not curl from the CPE surface. The electrode was left in a fume cupboard for drying under ambient condition for about 1 hour. Then, 10 μl of stock sol-gel solution was pipetted to cover the enzyme modified CPE. The stock sol-gel solution on the CPE was allowed to polymerize and dried for 1 hour at room temperature. Finally, the enzyme electrode was immersed in a phosphate buffer (pH 7.0) and kept at 4°C in a refrigerator overnight. The purpose of soaking the electrode was to wash out of the excess HRP from the electrode surface. From our experience, a newly fabricated enzyme electrode used in the same day did not have a reproducible current response. When not in use, the electrode was also stored in a phosphate buffer (pH 7.0) at 4°C in a refrigerator.

2.3. Instrumentation

Cyclic voltammetric and amperometric measurements were performed using Echem Data System, including both hardware and software supplied by ADInstruments, Australia. The software was driven by an LC575 Macintosh computer. The three-electrode system consisted of a sol-gel immobilized HRP biosensor as working electrode, a Ag/AgCl (vs. sat. KCl) reference electrode and a platinum wire as auxiliary electrode. Experiments were carried out in an electrochemical cell holding 10 ml of deoxygenated 0.02 M phosphate buffer (pH 7.0) containing 50 mM ferrocyanide at 25.0±0.5°C. Cyclic voltammetric

experiments were performed in an unstirred solution. The voltammograms were recorded in the potential range of 0 to +400 mV and the scan rate was 25 mV s^{-1}. A magnetic stirrer and stirring bar provided the convective transport for the amperometric experiments. The stirring rate was fixed at 250 rpm. A holding potential of -100 mV was applied on the working electrode and background current was allowed to decay to a steady-state. Then, aliquots of hydrogen peroxide standard solution were added into the cell and current-time curves were recorded.

The flow injection analysis (FIA) experiments were carried out using Hewlett Packard HPLC model 1050 system detector. A 25 µl sample loop and a thin-layer cell were used. Amperometric experiments for FIA were performed by applying a potential of -100 mV vs. Ag/AgCl on the working electrode. This enzyme working electrode has a diameter of 4 mm and fabricated from Teflon. The carrier solution consisted of deoxygenated 0.02 M phosphate buffer (pH 7.0) solution containing 50 mM ferrocyanide and the flow rate of the carrier solution was 0.5 ml min^{-1}.

3. Results and discussion

3.1. Effect of the sol-gel film thickness on the enzyme electrode response

The response of the enzyme electrode depends on the mass transport of the mediator and substrate or product through the sol-gel film. The effect of the thickness of sol-gel film was studied by carrying out the calibration plots for hydrogen peroxide using different enzyme electrodes with varying sol-gel film thickness. In our previous work on sol-gel modified glucose electrode, the thickness of the sol-gel film had a profound effect on the electrode response [23]. The thickness of the sol-gel film was adjusted by varying the amount of methanol in the stock sol-gel solution, while the volumes of other compounds were fixed. The surface of the sol-gel modified enzyme electrode appeared to

be entirely smooth and flat. Any extremely fine cracks on the surface of the sol-gel film were not visible to the naked eyes.

It is evident from Fig. 1 that when the volume of methanol is 600 μl in the stock sol-gel solution, the enzyme electrode gives a maximum response with a wide dynamic range. The thin sol-gel film prepared using dilute stock sol-gel solution (volume of methanol was high) was not effective to immobilize HRP, leading to a low enzyme loading on the electrode surface. Thus, the calibration graph has very limited dynamic range and low sensitivity. On the other hand, when the volume of methanol in the stock sol-gel solution was too low, this produced a thick sol-gel film. The thick film could hinder part of the active centre of HRP, thus lowering the interaction of the substrate with HRP active centre, and consequently a low response of the enzyme electrode was obtained. In addition to that, the mediator and substrate or product experienced a greater diffusion barrier. Also, when the sol-gel film was thick, the enzyme electrode response was slow. The response time for 0.2 mM H_2O_2 for electrodes (a), (d) and (f) of Fig. 1 are 50, 30 and 20 sec respectively. It is clear that the response time of the enzyme electrode increased with the thickness of the sol-gel film thickness. The present optimized sol-gel film on the enzyme electrode was very thin, probably the thinnest sol-gel film ever reported on the sol-gel modified enzyme electrodes for electrochemical studies, this was not likely to hinder the diffusion of the mediator and substrate or product through the film. Thus, a fast response time was obtained both for amperometric and FIA experiments (Section 3.5).

3.2. Cyclic voltammetry

Fig. 2 shows the cyclic voltammograms obtained with the enzyme electrode in an unstirred 0.02 M deoxygenated phosphate buffer (pH 7.0) containing 1 mM ferrocyanide without H_2O_2 (a) and with 1 mM H_2O_2 (b). In the absence of H_2O_2, the HRP enzyme electrode contributes no response and only the electrochemical behaviour of ferrocyanide in

solution is observed (Fig. 2a). The redox behaviour of ferrocyanide at the enzyme electrode shows a reversible electrochemical response with $\Delta E_p = 56$ mV, which is close to the theoretical peak separation for one electron reaction. Upon addition of 1 mM H_2O_2, the voltammogram changes, with an increase in the reduction current and no increase in the oxidation current (Fig. 2b). The fact that the oxidation current does not increase along with the reduction current is indicative of the enzyme-dependent catalytic oxidation of the ferrocyanide to ferricyanide, which is reduced at the enzyme electrode subsequently.

3.3. Optimization of experimental variables

The various experimental parameters which can affect the amperometric determination of H_2O_2 are the concentration of the mediator, applied potential and the pH of the solution. The effect of applied potential on the enzyme electrode response is shown in Fig. 3. It was found that the sensitivity of the enzyme electrode increased slightly with increasing potential from -300 to -100 mV and decreased upon changing the potential from -100 to +200 mV. The increased sensitivity with applied potential can be attributed to the increased driving force for the reduction of ferricyanide which is produced during the course of the enzymatic reaction. The potential of -100 mV was selected for the remainder experiments so as to achieve the optimum sensitivity. Moreover, this low potential is favourable and expected to minimise possible interferences.

Fig. 4 illustrates the response of the enzyme electrode increases sharply as the concentration of the mediator, ferrocyanide increases and then levels off at a concentration of 50 mM. The dynamic range of the calibration curves was extended when the concentration of the mediator increased. Such a phenomenon is typical for a mediator-based sensor [5-6, 9]. When the mediator concentration was low (Fig. 4 a to c), the response will be limited by the enzyme-mediator kinetics. When the mediator concentration was high (Fig. 4 d to e), the response will be limited by enzyme-substrate kinetics. Higher

concentration of mediator was not selected because the background current also increased. Therefore, 50 mM ferrocyanide was used in this study.

The pH dependence of the enzyme electrode over the pH range of 5.5 to 8.5 in 0.02 M phosphate buffer in the presence of 0.6 mM H_2O_2 is illustrated in Fig. 5. The resulting peak-shaped pH profile showed a maximum sensitivity of the enzyme electrode at pH 7.0. This pH is close to the optimum pH 7.0 observed for soluble peroxidase [24]. The sol-gel matrix does not alter the optimum pH for the catalytic behaviour of the peroxidase. We selected pH 7.0 which is closer to the physiological pH, for this study even though the stability of ferrocyanide in solution increases at higher pH.

The amperometric experiments were carried out in solutions stirred under a fixed stirring rate of 250 rpm. It was observed that the response increased with the stirring rate and this can be explained by the thin diffusion layer generated at the electrode surface. When the stirring rate was very slow, the response took a long time to achieve equilibrium and the noise was very high. Meanwhile, when the stirring rate was too high, some bubbles were generated in the solution and the noise also increased.

3.4. Electrode response characteristics

Calibration and reproducibility of the enzyme electrode

Fig. 6 displays a typical current-time response obtained using the enzyme electrode under the optimized experimental conditions. The resulting calibration range of $2x10^{-4}$ to $3.29x10^{-3}$ M is presented in the inset of Fig. 6. It can be seen that rapid and sensitive response to changes in the concentration of H_2O_2 was achieved. The enzyme electrode exhibits a linear calibration range from $2x10^{-5}$ to $2.6x10^{-3}$ M with a slope of 52.17μA mM^{-1} and a correlation coefficient of 0.999. The apparent Michaelis-Menten constant (K_m^{app}), which gives an indication of the enzyme-substrate kinetics, for the enzyme electrode can be

calculated from the linear part of the calibration graph (Fig. 6), using the electrochemical Lineweaver-Burk equation [25]:

$$\frac{1}{I_{ss}} = \frac{1}{I_{\max}} + \frac{K_m^{app}}{I_{\max}} \frac{1}{c}$$

where I_{ss} is the steady state current after the addition of substrate, c is the bulk concentration of the substrate and I_{max} is the maximum current measured under saturated substrate conditions. Analysis of the slope and intercept for the plot of the reciprocal of the steady state current versus reciprocal of H_2O_2 concentration allows the determination of K_m^{app}. The K_m^{app} value for the enzyme electrode was found to be 4.8 mM. This K_m^{app} value is smaller than the reported K_m^{app} by Qian et al. for a peroxidase electrode [26] which implies that the present enzyme electrode exhibits a higher affinity for H_2O_2. The reaction was first order with respect to the substrate concentration when the substrate concentration was in the calibration linear range. The response was saturated at about 3.0 mM H_2O_2. The response at high substrate concentration can be attributed to the saturation of enzyme-substrate or enzyme-mediator kinetics.

The reproducibility of the enzyme electrode was investigated at a H_2O_2 concentration of 0.6 mM and the mean current was 32.5 μA with a relative standard deviation of 2.1% for n=10. The fabrication reproducibility of six electrodes, independently made, showed an acceptable reproducibility with a relative standard deviation of 3.9% for the current obtained in 0.6 mM H_2O_2.

Selectivity against interferences

Electroactive interferences have been a problem when biological or industrial fluids are assayed by amperometric biosensors. The effect of substances that might interfere with the response of the enzyme electrode was examined. The current obtained for each interfering substance at a concentration of 1.2 mM (except otherwise stated) in the presence

of 0.6 mM H_2O_2 is compared to the current obtained in the presence of 0.6 mM H_2O_2 alone as a criterion for the selectivity of the electrode. Table 1 summarizes the interfering effects among various species tested. Glucose, sucrose, ethanol, acetic acid, citric acid, oxalic acid, nitrate do not cause any observable interference to the determination of H_2O_2. Of the interferences studied, only ascorbic acid and S^{2-} ion (0.2 mM) interfered to a significant extent and F^- ion interfered slightly. Ascorbic acid can reduce the ferricyanide produced in the enzymatic reaction of peroxidase on H_2O_2 and thus interferes with the H_2O_2 determination. The S^{2-} ion interferes with the H_2O_2 determination by inhibiting the activity of the HRP and reducing ferricyanide produced. This observation is consistent with the previous report [5].

Thermal and storage stability

The thermal stability of the enzyme electrode was studied by the following procedure: The initial response of the electrode was obtained in the presence of 1 mM H_2O_2 at 25°C. Then, after having immersed the enzyme electrode in a buffer solution at a certain temperature for 1 hour, the response of the electrode was again obtained at 25°C in the presence of 1 mM H_2O_2. The ratio of currents for the electrode after immersing at a high temperature with before immersing is evaluated. Table 2 shows the results of the thermal stability of the enzyme electrode. It can be seen that the thermal stability decreased with increasing temperature due to the denaturation of the enzyme. Therefore, the enzyme electrode was kept at 4°C in refrigerator between experiments.

The storage stability of the enzyme electrode was examined by calibration of the sensor with standard H_2O_2 solution. Fig. 7 shows the calibration graphs of H_2O_2 after 5, 15, 25, 35 days of storage with the calibration graph of a newly made enzyme electrode. The sensitivity and the linear dynamic range of the electrode decrease gradually with storage period. After 35 days of storage, the enzyme electrode in a phosphate buffer at

4°C, this electrode has about 60% of the original sensitivity. It is obvious that sol-gel immobilization technique is efficient to retain the activity of HRP. The sol-gel film acts as a protective layer on CPE preventing it from swelling which is an added advantage of this immobilization technique. This protective function is especially advantageous in flowing solutions.

3.5. FIA experiments

FIA was performed using this enzyme electrode as electrochemical detector in a thin layer cell and typical flow injection peaks are shown in Fig. 8. The potential of the working electrode was fixed at -100 mV. Fig. 8 (a) and (b) compare the sensitivity of unmodified CPE (a) and sol-gel modified HRP CPE (b) for the concentrations of 0.8 mM (1) and 6 mM (2) of H_2O_2 respectively. The sensitivity of the modified enzyme electrode increased about 30 times (Fig. 8b) compared to the bare unmodified CPE (Fig. 8a). Fig. 8c gives the results of 8 samples of 0.6 mM H_2O_2 injected successively which indicates good reproducibility of the enzyme electrode. Moreover, it was observed that the sol-gel immobilized enzyme electrode did not swell in the flowing solution after a continuous operation which implies the stability of the electrode whereas the bare CPE swelled after a brief operation in the flowing solution. The calibration of the enzyme electrode performed under FIA is $2x10^{-5}$ to $4x10^{-3}$ M H_2O_2, which is slightly wider than calibration plot obtained using amperometric method.

4. Conclusions

We report that HRP is successfully immobilized by the sol-gel technique on a carbon paste electrode which functions as a selective and sensitive H_2O_2 biosensor. The H_2O_2 biosensor is based on the mediator ferrocyanide shuttling electrons between the HRP in the silica sol-gel matrix and the carbon paste electrode. The low operating potential for

the enzyme electrode is an advantage to reduce interferences from other interfering substances. The enzyme electrode exhibits a remarkable electrochemical response in terms of stability, sensitivity and reproducibility, since the sol-gel immobilized HRP CPE not only increases the sensitivity for H_2O_2 detection but also improves the stability of the unmodified CPE. Therefore, it is suitable to be used as an on-line electrochemical detector in FIA. The results clearly demonstrate that sol-gel technique is an attractive approach for immobilization of enzymes which can be employed for electrochemical biosensors, and furthermore, for a wide range of other biosensors.

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Legend

Figure 1:

The effect of the thickness of sol-gel film on the enzyme electrode response studied by calibration plots of H_2O_2 using amperometric measurement. Supporting electrolyte: 0.02 M phosphate buffer (pH 7.0) containing 50 mM ferrocyanide. The volume of the methanol in the sol-gel stock solution (a) 100 μl, (b) 250 μl (c) 450 μl, (d) 600 μl, (e) 700 μl, and (f) 1000 μl. Operating potential -100 mV vs. Ag/AgCl.

Figure 2:

Cyclic voltammograms of sol-gel HRP-modified enzyme electrode in 0.02 M phosphate buffer (pH 7.0) containing 1 mM ferrocyanide (a) without and (b) with 1 mM H_2O_2. Scan rate 25 mV s^{-1}.

Figure 3:

Effect of potential on the enzyme electrode response studied by amperometric method for 0.6 mM H_2O_2 in 0.02 M phosphate buffer (pH 7.0) containing 50 mM ferrocyanide. Operating potential -100 mV vs. Ag/AgCl.

Figure 4:

Influence of the mediator concentration on the dynamic response range of enzyme electrode response studied by amperometric method in 0.02 M phosphate buffer (pH 7.0). Concentration of ferrocyanide mediator (a) 1 mM, (b) 5 mM, (c) 10 mM, (d) 50 mM and (e) 100 mM. Operating potential -100 mV vs. Ag/AgCl.

Figure 5:

Effect of pH on the enzyme electrode response studied by amperometric method for 0.6 mM H_2O_2 in 0.02 M phosphate buffer (pH 7.0) containing 50 mM ferrocyanide. Operating potential -100 mV vs. Ag/AgCl.

Figure 6:

Typical current-time response curve for successive additions of H_2O_2 for the enzyme electrode in 0.02 M phosphate buffer (pH 7.0) containing 50 mM ferrocyanide. Operating potential -100 mV vs. Ag/AgCl. Inset shows a resulting calibration curve.

Figure 7:

The effect of storage time on the enzyme electrode response studied by calibration plots of H_2O_2 using amperometric measurement. Supporting electrolyte: 0.02 M phosphate buffer (pH 7.0) containing 50 mM ferrocyanide. Operating potential -100 mV vs. Ag/AgCl.

Figure 8:

FIA peaks for 0.8 mM (1) and 6 mM (2) of H_2O_2 at the bare unmodified CPE (a), and modified enzyme electrode (b) respectively. (c) FIA peaks for 0.6 mM H_2O_2 for 8 eight successive injections. Experimental conditions: carrier solution 0.02 M phosphate buffer (pH 7.0) containing 50 mM ferrocyanide. Operating potential -100 mV vs. Ag/AgCl. Flow rate 0.5 ml min^{-1}.

List of Tables

Table 1 Possible interferences tested with the enzyme electrode

Table 2 The thermal stability of the silica sol-gel modified HRP electrode

Table 1 Possible interferences tested with the enzyme electrode

Interfering substance	Current ratios*
glucose	1.03
sucrose	1.00
ethanol	1.00
acetic acid	1.00
citric acid	0.99
oxalic acid	1.04
ascorbic acid	0.61
NO_3^-	0.98
S^{2-} (0.2 mM)	0.18
F^-	0.92

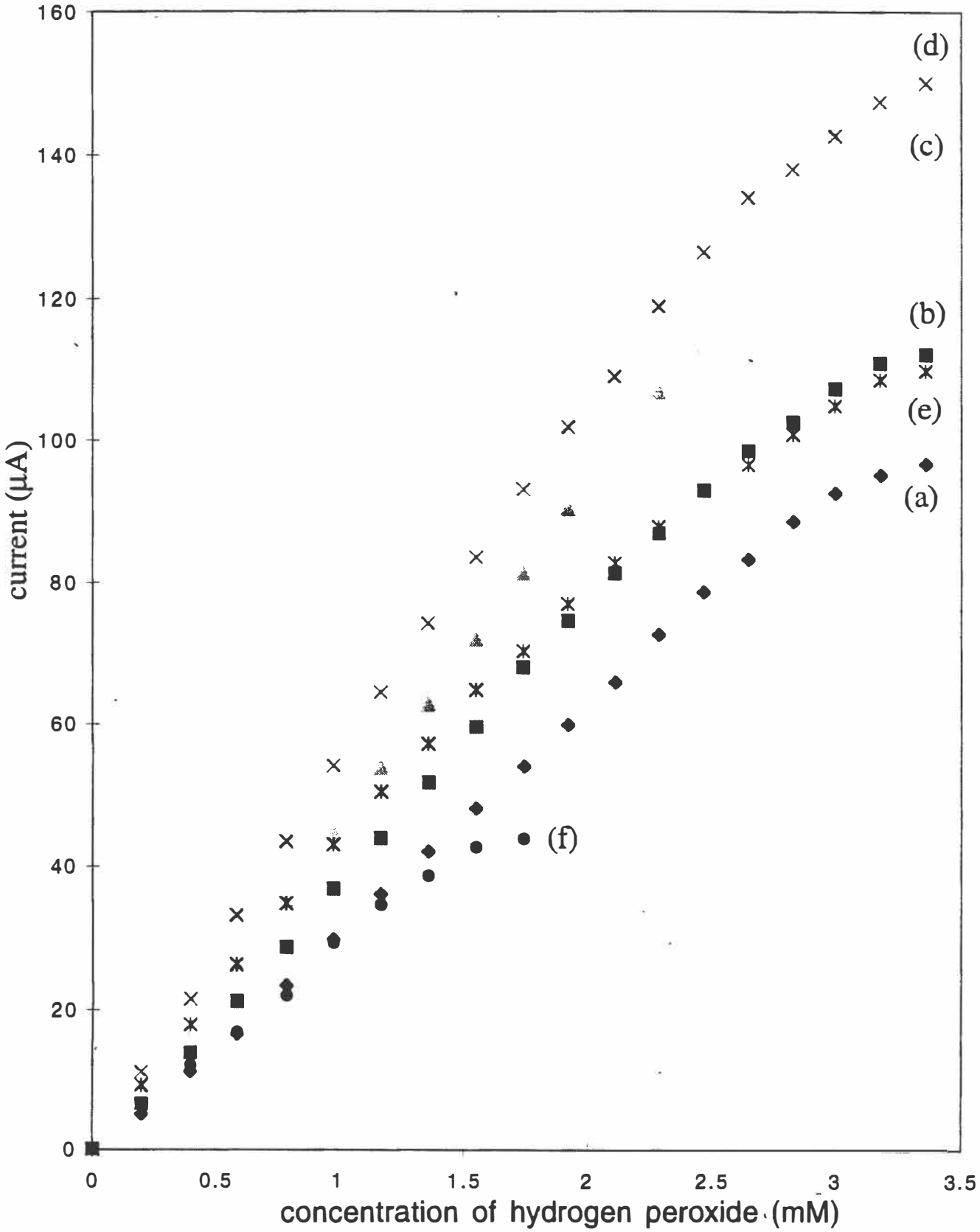
* Ratio of currents for mixtures of 1.2 mM interfering substance and 0.6 mM H_2O_2 compared to that for 0.6 mM H_2O_2 alone.

Table 2 The thermal stability of the silica sol-gel modified HRP electrode

temperature (°C)	25	35	45	55	65
current ratios#	0.98	0.91	0.86	0.50	0.25

Ratio of currents for the enzyme electrode response after immersing in a specific temperature for 1 hour compared to the response before immersing at 25°C.

Figure 1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140
120
100
80
60
40
20
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current (μA)
0
0.5
1
1.5
2
2.5
3
3.5
concentration of hydrogen peroxide (mM)
(d)
(c)
(b)
(e)
(a)
(f)

Figure 2

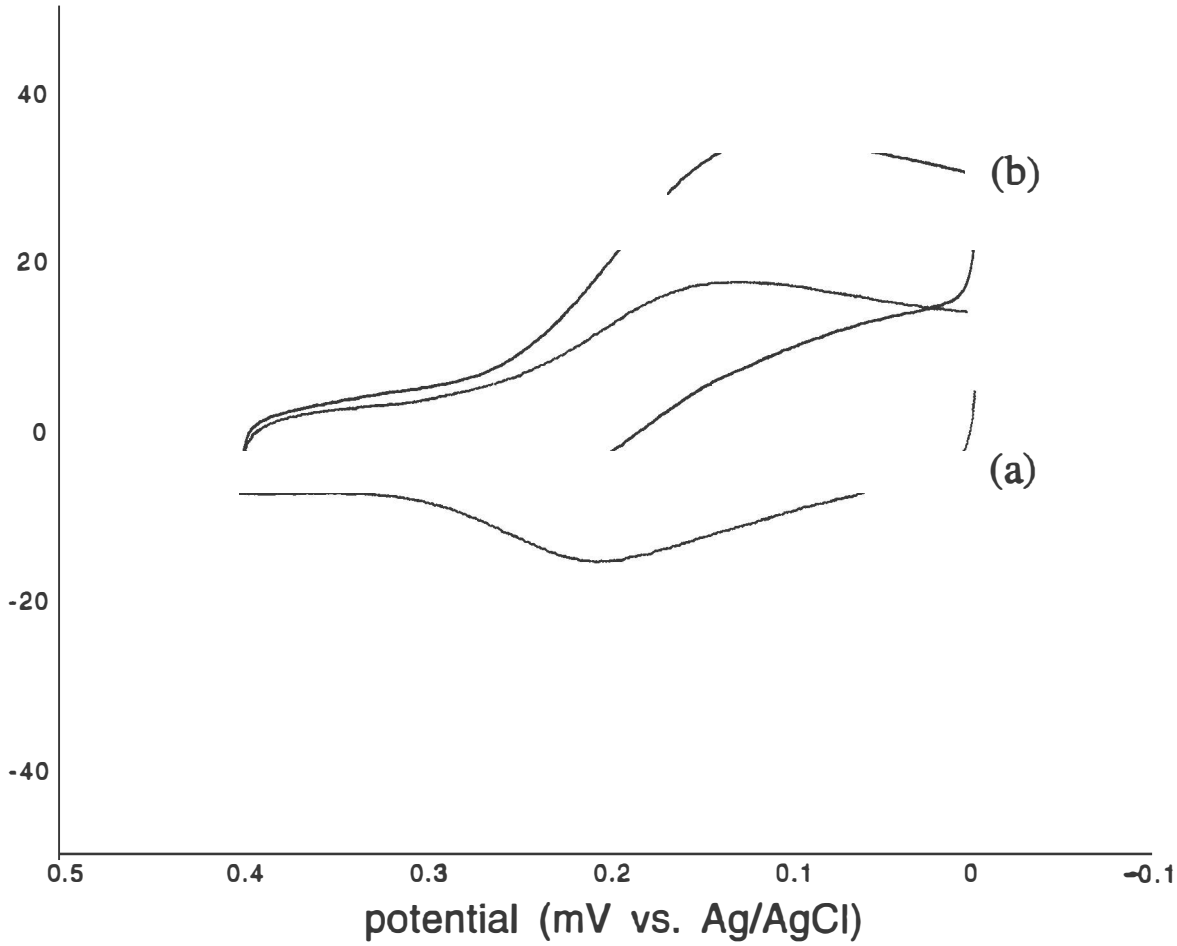
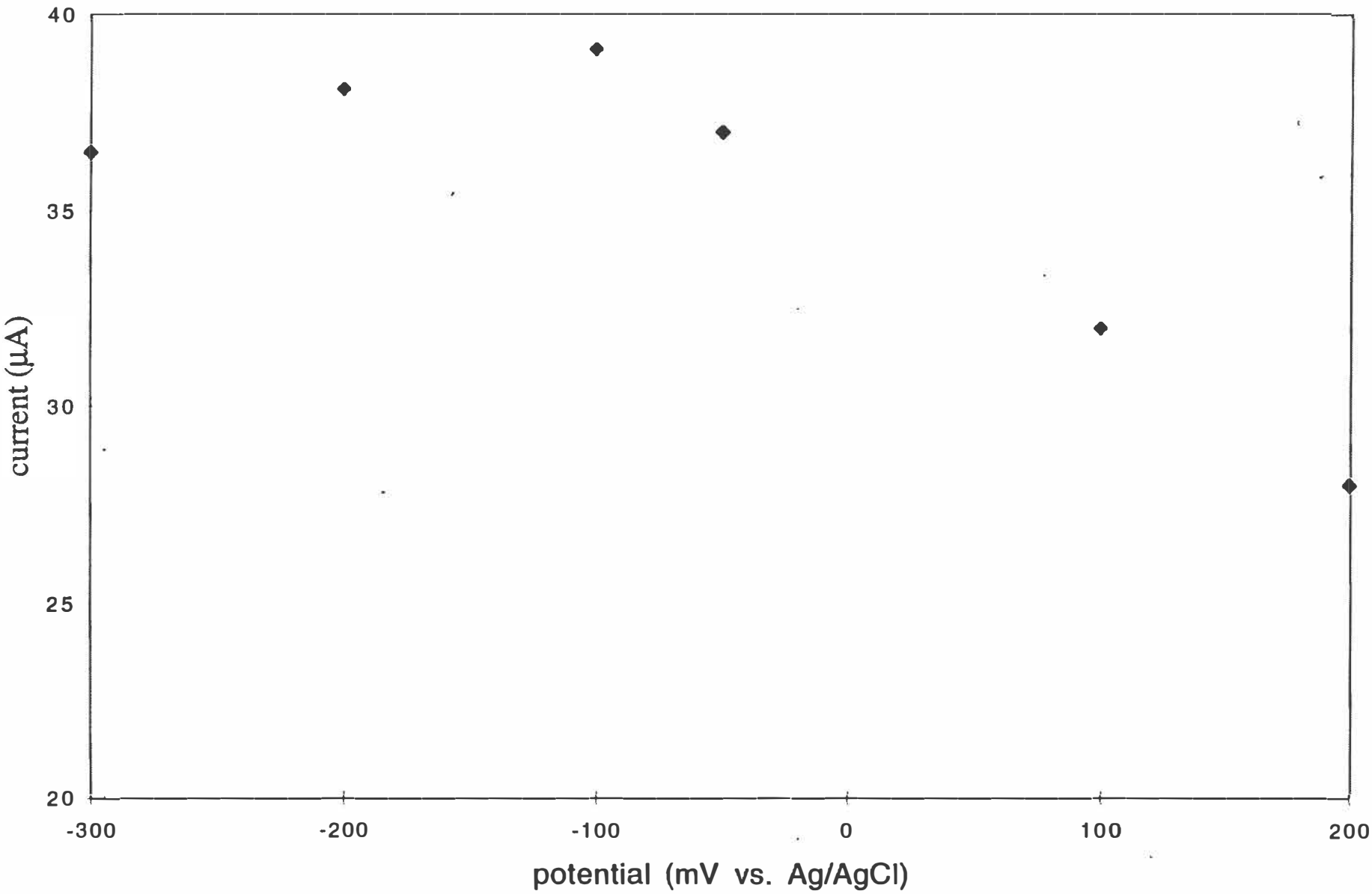


Figure 3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25
20
current (µA)
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-200
-100
0
100
200
potential (mV vs. Ag/AgCl)

Figure 4

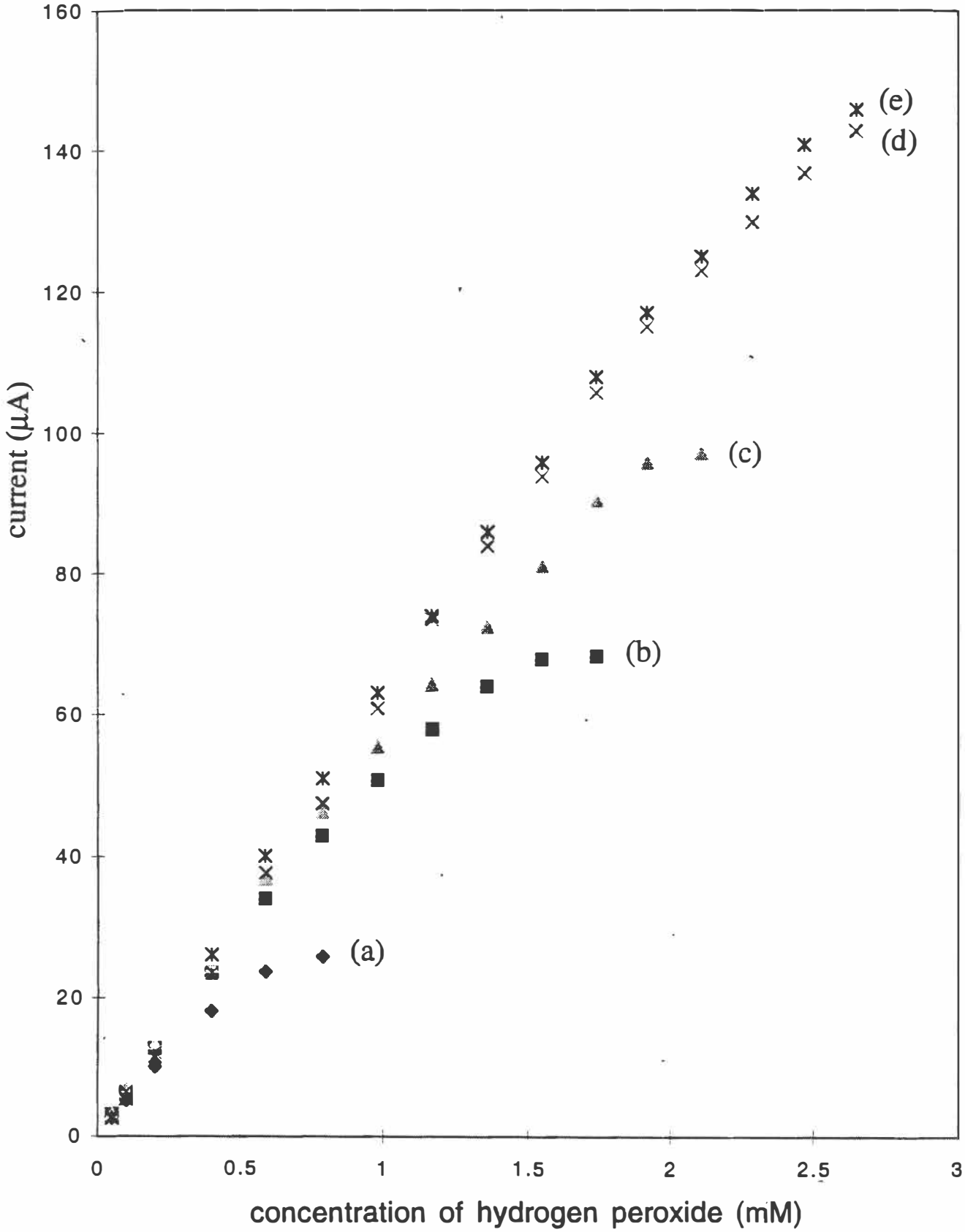

160
140
120
100
80
60
40
20
0
current (μA)
0
0.5
1
1.5
2
2.5
3
concentration of hydrogen peroxide (mM)
(a)
(b)
(c)
(d)
(e)

Figure 5

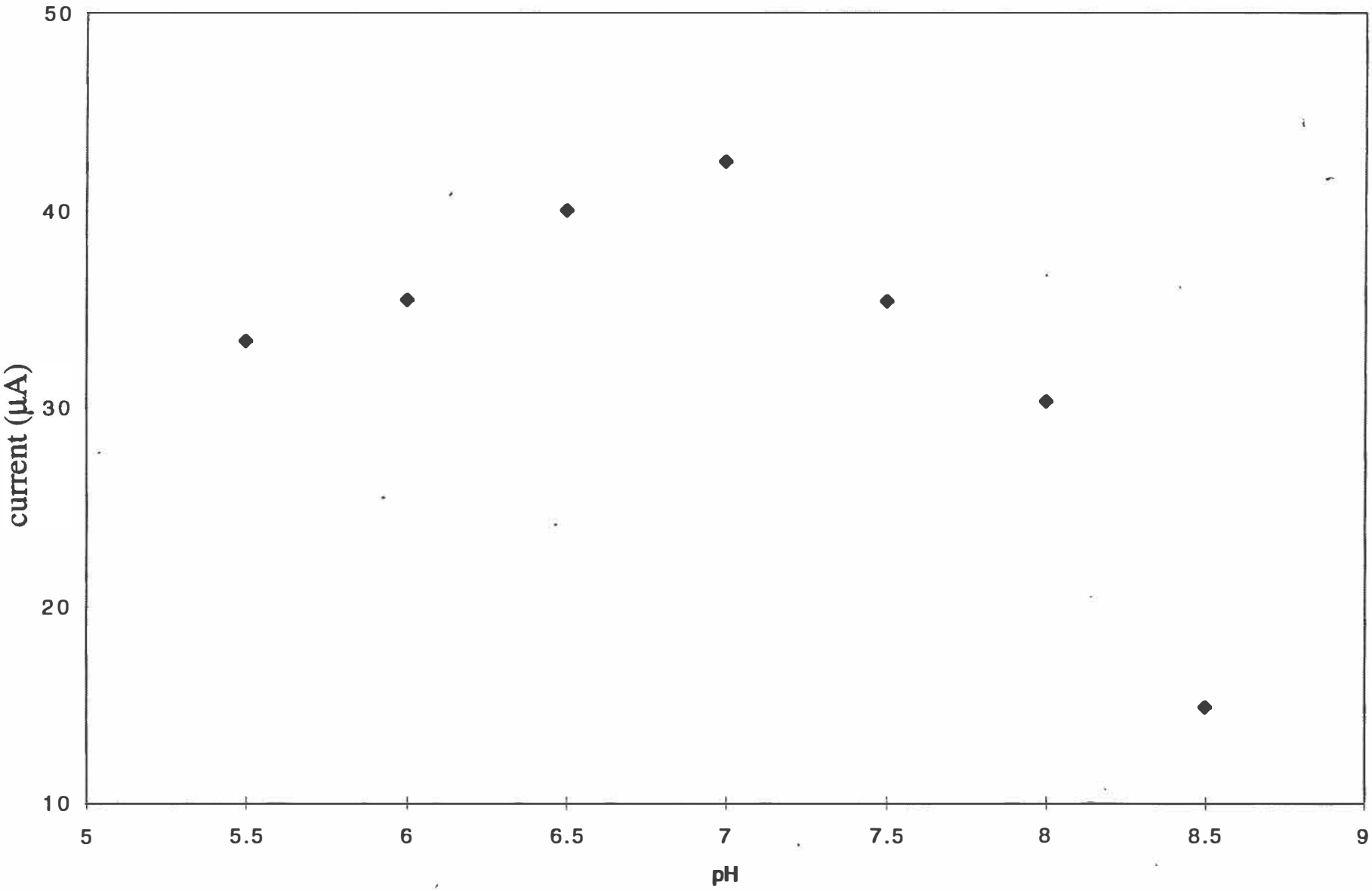


Figure 6

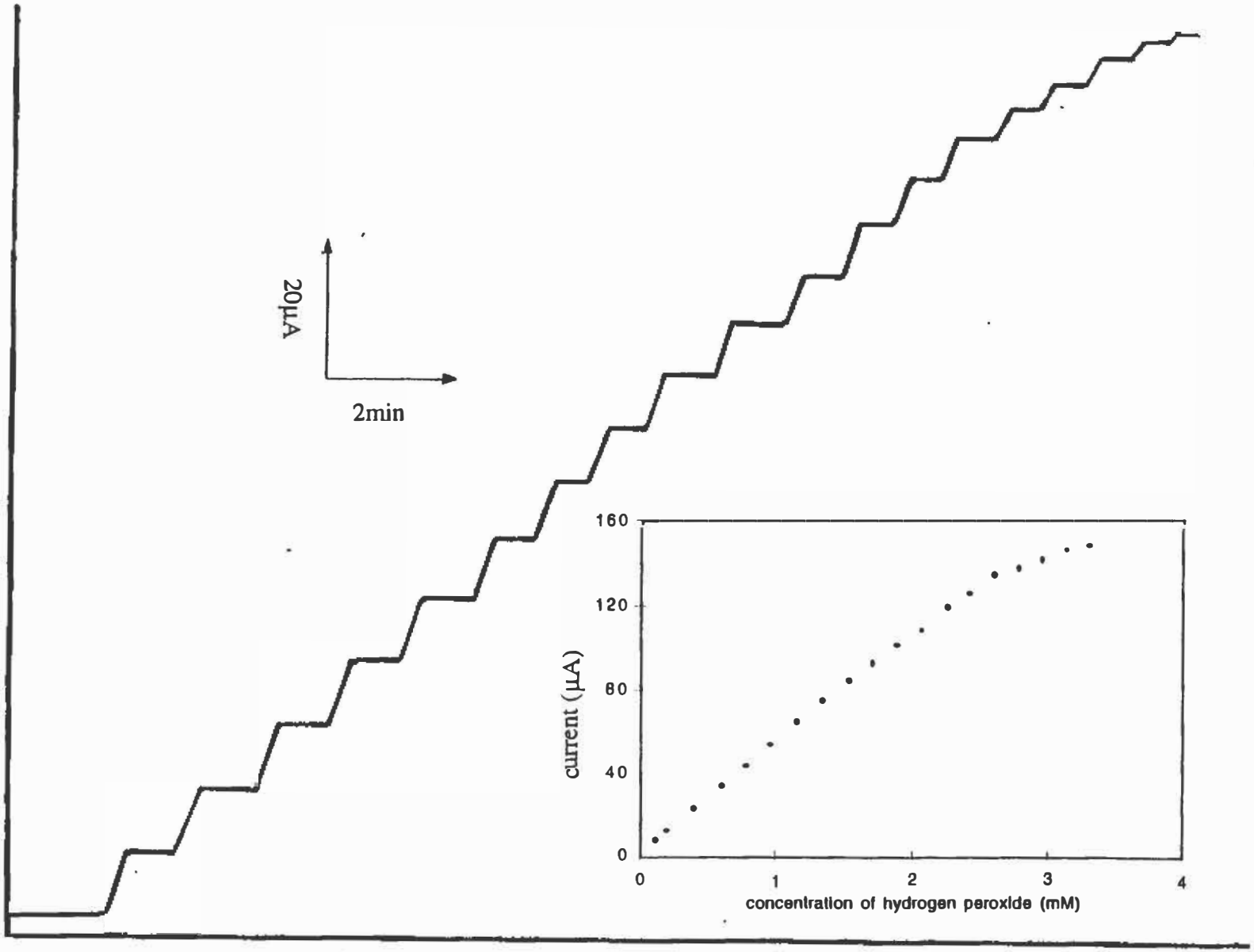
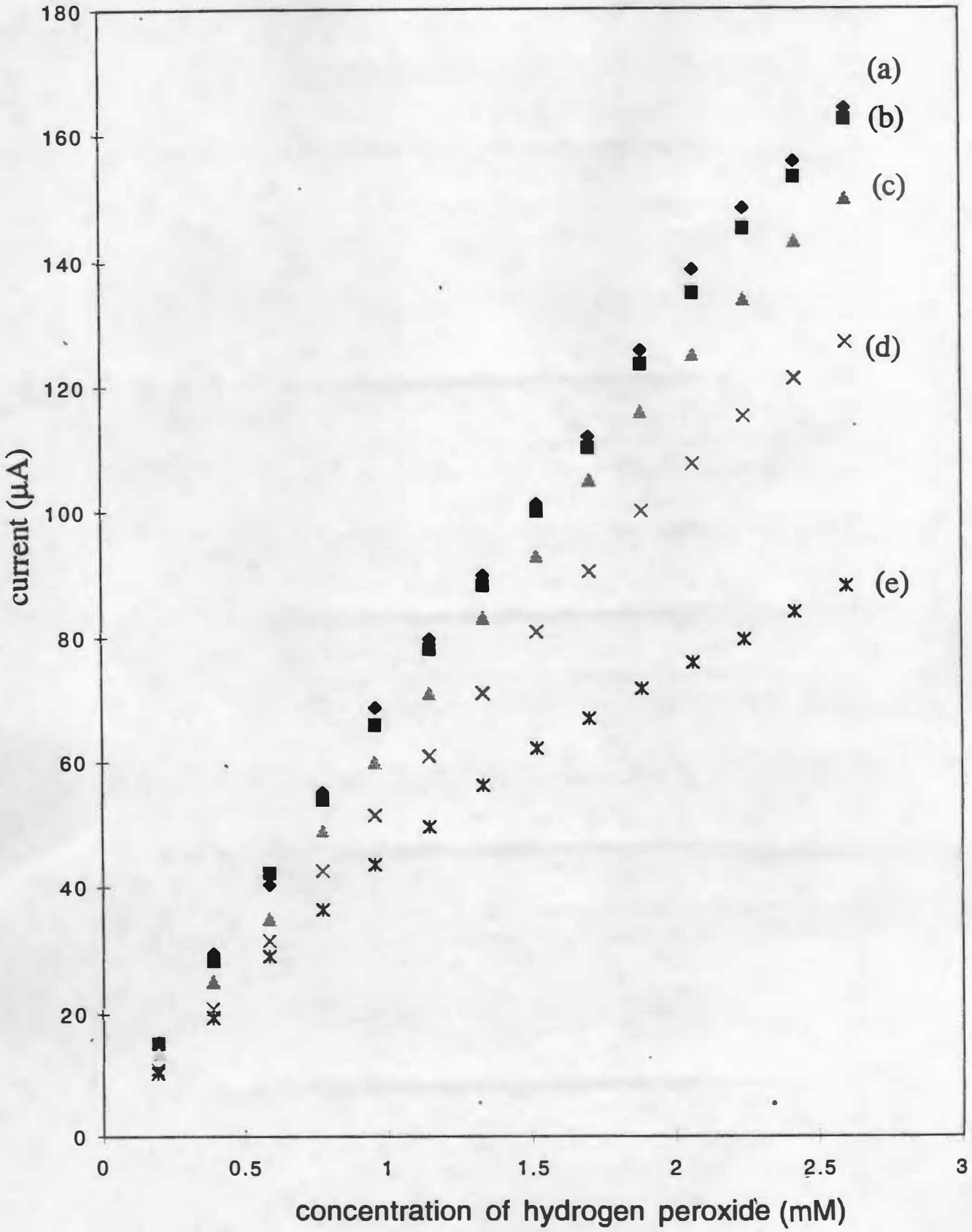

20μA
2min
160
120
80
40
0
current (μA)
0
1
2
3
4
concentration of hydrogen peroxide (mM)

Figure 7


180
160
140
120
100
80
60
40
20
0
current (μA)
0
0.5
1
1.5
2
2.5
3
concentration of hydrogen peroxide (mM)
(a)
(b)
(c)
(d)
(e)

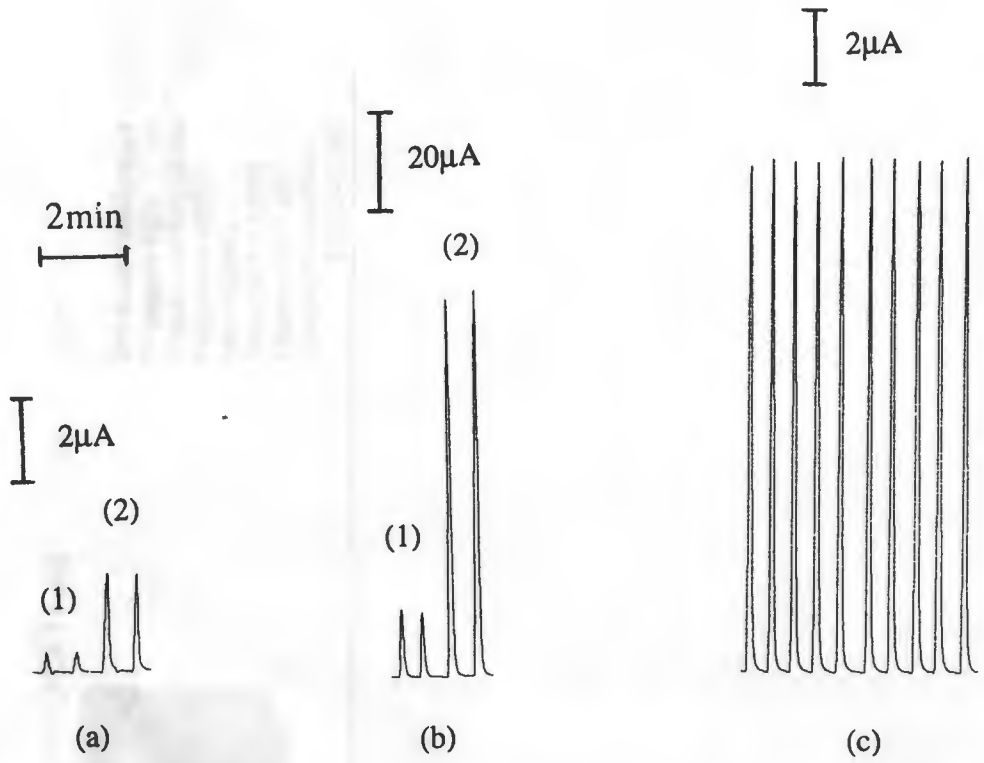



Figure 8