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Multienzymatic-rotating biosensor for total cholesterol determination in a FIA system

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Abstract

Fabrication of an amperometric-rotating biosensor for the enzymatic determination of cholesterol is reported. The assay utilizes a combination of three enzymes: cholesterol esterase (ChE), cholesterol oxidase (ChOx) and peroxidase (HRP); which were co-immobilizing on a rotatory disk. The method is developed by the use of a glassy carbon electrode as detector versus Ag/AgCl/3 M NaCl in conjunction with a soluble-redox mediator 4-tert-butylcatechol (TBC). ChE converts esterified cholesterol to free cholesterol, which is then oxidized by ChOx with hydrogen peroxide as product. TBC is converted to 4-tert-butylbenzoquinone (TBB) by hydrogen peroxide, catalyzed by HRP, and the glassy carbon electrode responds to the TBB concentration. The system has integrated a micro packed-column with immobilized ascorbate oxidase (AAOx) that works as prereactor to eliminate L-ascorbic acid (AA) interference. This method could be used to determine total cholesterol concentration in the range 1.2 μM–1 mM ($r = 0.999$). A fast response time of 2 min has been observed with this amperometric-rotating biosensor. Lifetime is up to 25 days of use. The calculated detection limits was 11.9 nM. Reproducibility assays were made using repetitive standards solutions ($n = 5$) and the percentage standard error was less than 4%.

Keywords: Cholesterol; Cholesterol esterase; Cholesterol oxidase; Horseradish peroxidase; Glassy carbon electrode; FIA

1. Introduction

The clinical analysis of cholesterol (Chol) in serum samples is important in the diagnosis and prevention of a large number of clinical disorders. There is a strong positive correlation between high serum Chol level and several illnesses as atherosclerosis and hypertension which can develop into coronary heart disease, myocardial and cerebral infarction (stroke).

In conditions such as hypothyroidism, nephrosis, diabetes mellitus, myxedema, and obstructive jaundice, the patient will have increased levels of Chol and its esters above the physiological norm. Decreased levels are found in patients suffering from hyperthyroidism, anemia, malabsorption and wasting syndromes.

Normal human blood serum contains less than 5.2 mM Chol (200 mg/dl), and a high level being considered as greater than 6.2 mM (240 mg/dl) [1,2]. Plasma Chol levels increase with age, and are generally less in women than men, until menopause, when the values in women exceed those in men [3]. Chol is carried in plasma by a series of protein-containing micelles known as lipoproteins. The lipoproteins are classified into distinct subtypes according to their density, very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate density lipoprotein (IDL) and high density lipoprotein (HDL). About 70% of total plasma Chol contained within lipoproteins is esterified by fatty acids. Hence, the concentration of free Chol within lipoproteins is approximately 1.0–2.2 mM (40–85 mg/dl) [4].

Historically, Chol was measured using non-enzymatic spectrometry, via the production of a colored substance, chiefly via cholestapolyenes and cholestapolyene carbonium ions (Liebermann–Burchard reaction). This method suffered from poor specificity, instability of the color reagent, standardization difficulties, the variable reactivity of esters and the unstable and corrosive nature of the reagents used [5,6]. The selectivity of the chemical reaction was improved with the introduction of

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the enzymes, cholesterol esterase (ChE) and cholesterol oxidase (ChOx).

The cholest-4-en-3-one can be reacted with 2,4-dinitrophenylhydrazine to produce a colored hydrazone [7], although the consumption of O_2 [8], or the production of H_2O_2 [4,9–11]. These are the easier methods of quantifying Chol spectrophotometrically, with the latter being the preferred method.

A number of Chol biosensors have been developed over the past 30 years. Examples of optical biosensors, which determine Chol enzymatically have been developed [12–15]. A fiber optic device has been developed by co-immobilizing ChOx and ChE on preactivated nylon membrane for free and total Chol estimations [16] and enzyme-based determination of Chol using the quartz crystal acoustic wave sensor also has been published [17].

The total Chol present in all lipoproteins fractions can be determined using amperometric and potentiometric assays specific for Chol [18–22]. Cholesterol and cholesterol esters are acted upon by cholesterol esterase and oxidase to generate H_2O_2, which could be measured amperometrically with modified electrodes [23–28].

In the present paper, we report the results of our systematic studies on the technical development and optimization of an amperometric-rotating biosensor. This is a multienzymatic system in which ChE, ChOx and horseradish peroxidase (HRP) are simultaneously immobilized on a disk rotatory. Detection is accomplished with the use of a glassy carbon electrode along with a soluble-redox mediator 4-tert-butylcatechol (TBC). ChE converts esterified cholesterol to free Chol, which is then oxidized by ChOx with hydrogen peroxide as product. TBC is converted to 4-tert-butyl-*o*-benzoquinone (TBB) by hydrogen peroxide, catalyzed by HRP, and the glassy carbon electrode responds to the TBB concentration. Besides, the system has integrated a micro packed-column with immobilized ascorbate oxidase (AAOx), that works as prereactor to eliminate L-ascorbic acid (AA) which is very important electroactive interferent.

2. Experimental

2.1. Reagents and solutions

All reagents used, except as noted, were of analytical reagent grade. Horseradish peroxidase (HRP, EC 1.11.1.7 Grade II, 181 IU mg^{-1}), cholesterol oxidase (ChOx, EC 1.1.3.6, from *Pseudonomas fluorescens*, 4.2 UI mg^{-1}), cholesterol esterase (ChE, EC 3.1.1.13 from *Pseudomonas* sp., 5.9 UI mg^{-1}), ascorbate oxidase (AAOx; 1000–3000 UI mg^{-1}), cholesterol and Triton X-100 were all purchased from Sigma (St. Louis, USA). A stock 0.01 M cholesterol solution was prepared in phosphate buffer (0.01 M, pH 7) containing 10% (w/w) of Triton X-100 in a thermostated bath at 65 °C. This solution was stored at 4 °C in the dark and was stable for at least 10–15 days (until turbidity was observed). More dilute working solutions of cholesterol were prepared by dilution of the stock solution using a 0.01 M phosphate buffer solution containing 1% (w/w) of Triton X-100. This solution was used as the carrier solution in the FIA system.

Synthetic Chol serum samples were prepared with the concentrations of interferents indicated in the text. Quality controls human serum (QC) with a lower, medium and higher (1.50 mM (QC1), 3.96 mM (QC2) and 7.77 mM (QC3)) concentrations, respectively (*Accutrol*™ Chemistry controls, A-2034, Sigma Diagnostics), were used. The kit for spectrophotometric determination of Chol (*colestat enzimatic*®) was purchased from Wiener Lab., Argentina, and was used accordance with manufacture instructions. 4-Tert-butylcatechol (4-TBC), ascorbic acid (AA), uric acid, lysine, lactate, glucose and glutaraldehyde used were purchased from Merk, Darmstadt. 3-Aminopropyl-modified controlled-pore glass, 1400 Å mean pore diameter and 24 m^2 mg^{-1} surface area, was from Electro-Nucleonics (Fairfield, NJ) and contained 48.2 μmol g^{-1} of amino groups.

Aqueous solutions were prepared using purified water from a Milli-Q-system and the samples were diluted to the desired concentrations using a 10 ml Metrohm E 485 burette.

2.2. Apparatus

The main bodies of the amperometric-rotating bioreactor were made of Plexiglas. The design of the flow-through chamber containing the rotating enzyme biosensor and the detector system was described previously [29]. Glassy carbon electrode is on the top of the rotating reactor. The rotating reactor is a disk of Teflon in which a miniature magnetic stirring bar (Teflon-coated Micro Stir bar from Markson Science Inc., Phoenix, AZ) has been embedded. Typically, a sensor disk carries 1.4 mg of controlled-pore glass on its surface. Rotation of the lower reactor was initiated by a laboratory magnetic stirrer (Metrohm E649 from MetrohmAGHerisau, Switzerland) and controlled with a variable transformer with an output between 0 and 250 V and maximum amperage of 7.5 A (Waritrans, Argentina).

Micro packed-column was made of Tygon tubing (2.0 cm long, 2.0 mm i.d.), it contained controlled-pore glass with AAOx immobilized. A pump (Gilson Minipuls 3 peristaltic pump, Gilson Electronics Inc., Middleton, WI) was used for pumping, sample introduction, and stopping of the flow. Fig. 1 illustrates

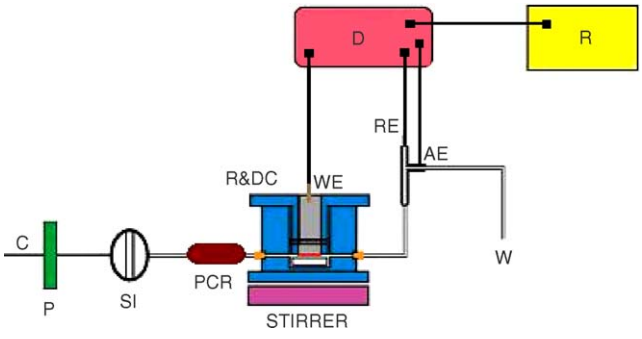


Fig. 1. Block diagram of the continuous-flow system and detection arrangement. P: pump (Gilson Minipuls 3 peristaltic pump, Gilson Electronics, Inc. Middleton, WI); C: carrier buffer line; SI: sample injection; W: waste line; R&DC: sensor and detector cell; WE: glassy carbon electrode; RE: reference electrode (Ag/AgCl/3.0M NaCl); AE: auxiliary electrode (platinum); D: potentiostat/detection unit (BAS LC-4C, Bioanalytical Systems, West Lafayette, IN); R: recorder (Varian, Model 9176, Varian Techtron, Springuale, Australia); PCR: packed-column reactor.

schematically the components of the single-line continuous-flow setup. The pump tubing was Tygon (Fisher AccuRated, 1.0 mm i.d., Fisher Scientific Co., Pittsburgh, PA) and the remaining tubing used was Teflon, 1.00 mm i.d. from Cole Parmer (Chicago, IL).

Amperometric detection was performed using a BAS LC-4C potentiostat and a BAS 100 B/W (Electrochemical Analyzer Bioanalytical System, West Lafayette, IN) was used to voltammetric determinations. The potential applied to the working electrode (Glassy carbon electrode, GCE) for the functional group detection was −150 mV versus Ag/AgCl/3.0 M NaCl reference electrode, BAS RE-6, and a Pt wire was used as counter electrode. At this potential, a catalytic current was well established.

Spectrophotometric measurements were performed with a Beckman DU 350 UV–vis spectrophotometer using 1 cm glass cells. All pH measurements were made with an Orion Expandable Ion Analyzer (Orion Research Inc., Cambridge, MA) Model EA 940 equipped with a glass combination electrode (Orion Research Inc., Cambridge, MA).

2.3. Enzymes immobilization

The rotating disk biosensor (bottom part) was prepared by immobilizing HRP, ChE and ChOx on 3-aminopropyl-modified controlled-pore glass (APCPG). The APCPG, smoothly spread on one side of a double-coated tape affixed to the disk surface, was allowed to react with an aqueous solution of 5% (w/w) glutaraldehyde at pH 10.00 (0.20 M carbonate) for 2 h at room temperature. After washed with purified water and 0.10 M phosphate buffer of pH 7.00, the enzymes mixture (constituted by HRP 40 U/ml, ChE 40 U/ml and ChOx 40 U/ml in 0.10 M phosphate buffer, pH 7.00) was coupled with residual aldehyde groups in phosphate buffer (0.10 M, pH 7.00) overnight at 4 °C.

By other way, AAOx was immobilized on 3-aminopropyl-modified controlled-pore glass (APCPG). The APCPG was allowed to react with an aqueous solution of 5% (w/w) glutaraldehyde at pH 10.00 (0.20 M carbonate) for 2 h at room temperature. After washing with purified water and 0.10 M phosphate buffer of pH 7.00, the enzyme (5 mg ml^{-1}) was coupled to the residual aldehyde groups in phosphate buffer (0.10 M, pH 7.00) overnight at 4 °C. Then the column was packed with 20 mg APCPG–AAOx preparation.

The both immobilized enzymes preparations (rotating disk and packed-column) were then washed with phosphate buffer (pH 7.00) and stored in the same buffer at 4 °C between uses. The immobilized preparations were perfectly stable throughout at least 1 month of daily use.

2.4. Validation

The samples from healthy human volunteers and Quality controls were diluted 1/10 before to carry out the measurements. Thus, the determinations were made in linear zone of the Chol calibration plot (see below).

To establish the lower limit of quantification in a single validation batch five replicates of QC sample 3.96 mM cholesterol were analyzed. The intra- and inter-day precision (CV%) and accuracy (bias%) of the assay procedure were determined by the analysis of five samples at each lower, medium and higher QC concentration (1.50 mM (QC1), 3.96 mM (QC2) and 7.77 mM (QC3)) in the same day and one sample at each QC concentration in five different days, respectively.

The freeze-thaw stability was also verified at two levels of concentrations, lower and higher, after three freeze-thaw cycles.

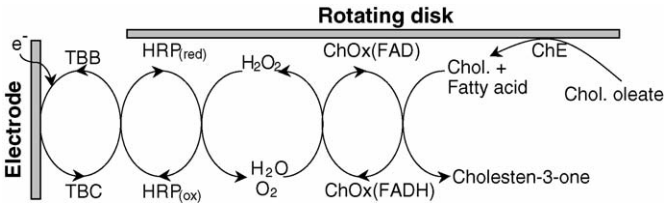
3. Results and discussion

The development of analytical system based on the use of immobilized multienzymes system represents one of the most rapid advancing areas of biosensors. Biosensors find a widespread application in scientific investigation as well as in medicine, biotechnological processes, food industries and environmental control.

The pioneering work of Kulys et al. [30] demonstrated that the combination of peroxidase and an oxidase makes possible to construct amperometric biosensors that work at more negative potentials avoiding electroactive interferences. This principle has also been used by other groups introducing an electroactive polymer to facilitate the HRP reduction [31,32]. However, most of the Chol in human blood is esterified with long chain fatty acids. Since cholesterol esters do not function as substrates for ChOx, biosensors based on ChE and ChOx have been developed for total Chol determination [33,34]. Cholesterol esterase catalyzes the hydrolysis of the esters to free cholesterol which can then become the substrate for ChOx oxidation. Cholesterol and cholesterol esters were acted upon by cholesterol esterase and oxidase to generate H_2O_2, which was measured amperometrically [23,35].

In our case, we have immobilized on the rotating disk HRP, ChOx and ChE in proportion 1:1:1, incorporating with the sample injection 4-TBC that works as mediator (Scheme 1). So the combination of 4-TBC and HRP, which reduces hydrogen peroxide, makes it possible to operate at a low potential and to eliminate interferents. The current developed at the detector is directly proportional to the concentration of analyte in the bulk of solution and increase with increasing rotation velocity.

Advantages of this configuration are the stability that results from immobilized enzymes in the rotatory disk and forced convection of the oxidized products to the electrode. If the flow is stopped when the sample plug transported by continuous-flow



Scheme 1. Schematic representations of the reduction wave of the enzymatic process between 4-tert-butylcatechol (TBC), 4-tert-butyl-*o*-benzoquinone (TBB), hydrogen peroxide (H_2O_2), cholesterol esterase (ChE), cholesterol oxidase (ChOx), and horseradish peroxidase (HRP).

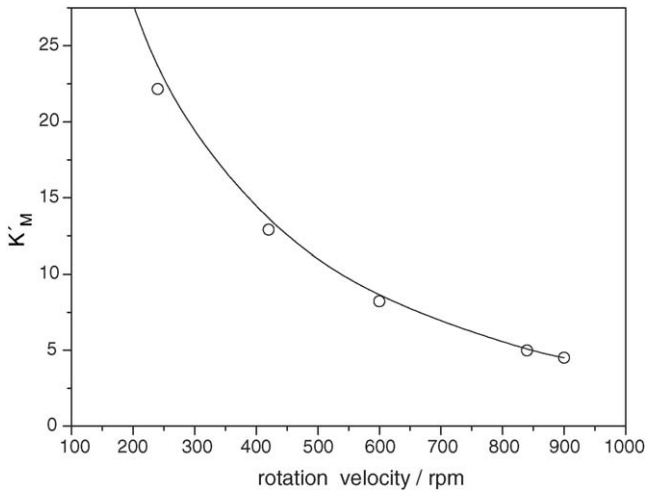


Fig. 2. The values of K'_M for the system obtained at five different rotation velocities and stopping the flow for 120 s during measurement. The calculation of K'_M was performed under conditions in which [substrate] > K'_M.

reaches the center of the reactor, detection take place under conditions similar to those of batch detection [36,37].

The implementation of continuous-flow/stopped-flow programming and the location of immobilized enzymes involved in the sequence illustrated earlier, permits: (a) utilization of relatively low enzyme loading conditions, (b) instantaneous operation under high initial rate conditions, (c) easy detection of accumulated products, and (d) reduction of apparent Michaelis–Menten constant, K'_M.

A more complete reagent homogenization is achieved, because the cell works as a mixing chamber by facilitating the arrival of substrate at the active sites and the release of products from the same sites. The net result is high values of catalytic current.

As noted at the beginning of this section, rotation is expected to decrease the values of the apparent Michaelis–Menten constant, K'_M, since the catalytic efficiency is increased. Michaelis constant which differ substantially from that measured in homogeneous solution, is not an intrinsic property of the enzyme, but of the system. This constant characterizes the reactor, not the enzyme itself. It is a measure of the substrate concentration range over which the reactor response is linear [38]. Fig. 2 shows the values of K'_M for the system obtained at five different rotation velocities and stopping the flow for 120 s during measurement. The calculation of K_M was performed under conditions in which [substrate] > K'_M, as a consequence the following applies (Eq. (1)), assuming that the Briggs and Haldane scheme [39] is of the plot of $1/S$ versus $1/[\text{Chol}_{\text{Total}}]$ plot. This is a graphical approach similar to the Lineweaver–Burk plot:

$$\frac{1}{S} = \left(\frac{m}{[\text{Chol}_{\text{Total}}]}\right) + n \quad (1)$$

where S = rate of response; $K'_M = m/n$. The apparent constant is thus obtained from the slope and intercept of the $1/S$ versus $1/[\text{Chol}_{\text{Total}}]$ plot.

Thereby, as observed earlier [40], if the sensor is devoid of rotation, there is practically no response. If a rotation of 900 rpm is imposed on the sensor located at the bottom of the cell (with immobilized enzymes), the signal is dramatically enlarged. The trend indicates that, up to velocities of about 900 rpm, a decrease in the thickness of the stagnant layer improves mass transfer to and from the immobilized enzyme active sites. Beyond 900 rpm, the current is constant, and chemical kinetics controls the overall process. Therefore, a rotation velocity of 900 rpm was used.

Advantages to these biosensors include rapid analysis, reusability, thermal stability, and linearity.

3.1. Effect of cell volume and sample size

Depending on the volume of the cell in contact with the sensors, the overall process becomes controlled by diffusion (large volumes) or by the chemical kinetics of the enzyme-catalyzed reactions (small volumes).

The cell volume was changed from 150 μl to 1 ml by removing the O-rings between the upper and lower half of the cell. The measured current, as expected, decreased linearly with an increase in cell volume, due to the dilution effect favored by rotation, and the fact that the measured current is directly proportional to bulk concentration. The smallest cell volume of 150 μl was adopted for further studies.

The measured current increased lineally with sample size up to 150 μl in a cell with a volume of 150 μl. For convenience a sample size of 150 μl was used. Sensitivity is almost tripled in the range between 50 and 150 μl.

3.2. Effect of pH

The catalytic current (nA), under stopped-flow conditions, presents a moderate increase from pH 6.5 to 7.00 and then begin to decay up to pH 8.00, the highest pH value tested (Fig. 3).

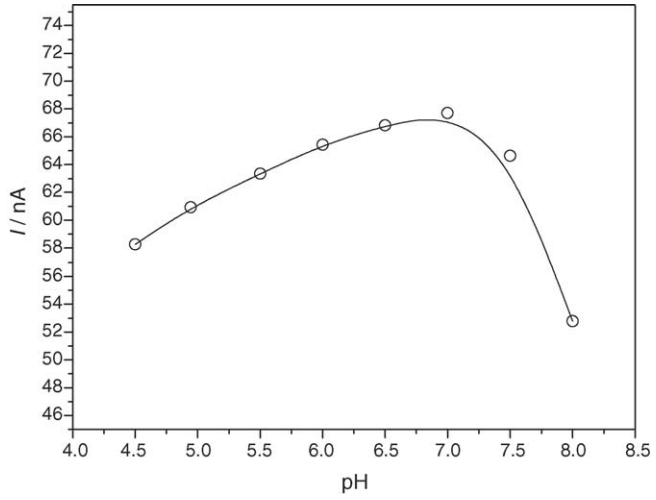


Fig. 3. pH dependence of multienzymatic-rotating biosensor signal. One hundred and fifty microliter aliquots of 3.96 mM Chol standard solution were injected into various buffers solutions. Other biosensor conditions were described in the text.

Therefore, the pH used for this system was 7.00 (0.10 M phosphate buffer).

3.3. Cholesterol measurement with multienzymatic-rotating biosensor

The working potential was selected using the cyclic voltammogram of 4-TBC at a glassy carbon electrode (GCE) in phosphate buffer [41]. For potentials values below −150 mV, the cathodic current became independent of the applied potential; therefore, this value was chosen as working potential. Furthermore, at this potential, a less contribution of the electroactive interferences present in serum is expected.

The performance of the multienzymatic-rotating biosensor for the measurement of total Chol concentration was characterized. The following procedure was used: (a) a baseline current was established with the buffer solution; (b) a solution containing the sample diluted 1/10 with buffer phosphate and 4-TBC was injected in the rotating biosensor; (c) the flow was detained, the disk was rotated to 900 rpm and the reduction current was measurement; (d) After 2 min the flow was started again. A Chol calibration plot was obtained by plotting catalytic current I (nA) versus Chol concentration. The results obtained with this method are shown in Fig. 4. A linear relation (Eq. (2)) was observed in the range of 1.2 μM and 1 mM (rotation 900 rpm):

$$I(\text{nA}) = 0.81 + 69.49[C_{\text{Chol}}] \quad (2)$$

The correlation coefficient for this type of plot was typically 0.998. Detection limit (DL) is the minimal difference of concentration that can be distinguished from the signal of baseline buffer solution. The DL was calculated as the amount of Chol required to yield a net peak that was equal to three times the SD signal. In this study, the minimal concentration of Chol was 11.9 nM. Reproducibility assays were made using repetitive standards solutions ($n = 5$) containing 1.0 mM 4-TBC and 3.96 mM Chol; the percentage standard error was less than 4%.

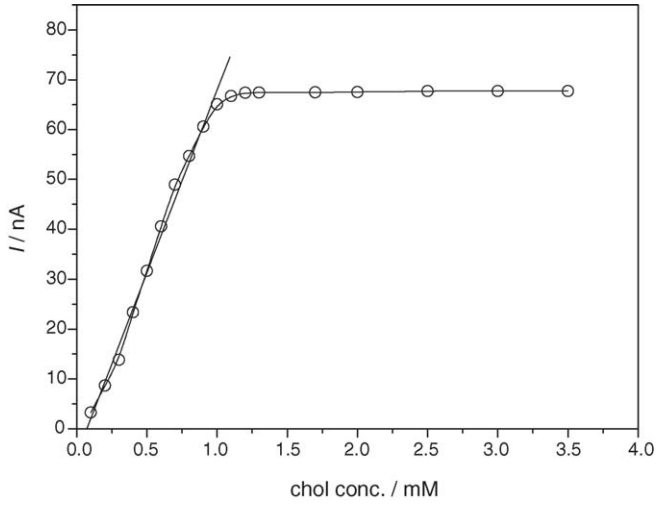


Fig. 4. Response of the multienzymatic-rotating biosensor for several Chol concentrations (mM). The flow was stopped for 2 min during measurement; cell volume: 150 μl; flow rate: 1 ml min^{-1}.

Table 1
Intra- and inter-day precision and accuracy ($n = 5$)

	Intra-day			Inter-day		
c_{nominal} (mM)	1.50	3.96	7.77	1.50	3.96	7.77
Mean c_{found} (mM)	1.47	3.93	7.72	1.39	3.83	7.59
CV (%)	0.70	1.10	1.80	1.00	1.40	2.30
Bias (%)	2.00	0.48	0.67	7.33	3.16	1.03

The stability of the biosensor was tested for nearly 3 h of continuous use in the FIA system. In this experiment, after every four samples, a standard solution containing 1.0 mM 4-TBC, 3.96 mM Chol was injected. In the FIA system using a multienzymatic sensor, there is practically no decay in the catalytic current after eight samples.

The effect of the various compounds on the response of the multienzymatic-rotating biosensor was tested. Compounds such as lysine, glucose, lactate and uric acid showed little or no effect on the current response. But the addition of 10 mM ascorbic acid resulted in ca. 4.8% decrease versus the reductive current obtained with Chol synthetic and 4-TBC. This fact can be avoided with the incorporation of a packed-column (PCR) that contains APCPG–AAOx preparation (Fig. 1).

The intra- and inter-day precision (CV%) and accuracy (bias%) of the assay procedure were determined by the analysis of five samples at each lower, medium and higher QC concentration in the same day and one sample at each QC concentration in five different days, respectively (Table 1).

Repeated analyses using a range of human serum samples, containing low and high levels of Chol demonstrate the precision of the Chol biosensor. This was found to range from 1.4 to 4.6% for within-day analyses and from 1.8 to 5.9% for day-to-day analyses. Recovery yields were performed using the clinical serum samples which were diluted with the working phosphate buffer and spiked with Chol standards. Recoveries in the range 96.6–103% with an average of 100.1% were obtained. The response study of this multienzymatic-rotating biosensor was compared with spectrophotometric methods. Table 2 gives the results obtained using the 2 methods for 5 separate determinations of 10 human serum samples.

Table 2
Results obtained using the 2 methods for 5 separate determinations of 10 human serum samples

Sample no.	Proposed method (mM)[a]	Reference method (mM)[a]
1	$3.96 \pm 4.1 \times 10^{-3}$	$3.90 \pm 6.1 \times 10^{-3}$
2	$3.83 \pm 3.6 \times 10^{-3}$	$3.89 \pm 5.2 \times 10^{-3}$
3	$1.50 \pm 3.1 \times 10^{-3}$	$1.56 \pm 4.1 \times 10^{-3}$
4	$1.46 \pm 2.9 \times 10^{-3}$	$1.40 \pm 3.9 \times 10^{-3}$
5	$7.80 \pm 3.9 \times 10^{-3}$	$7.69 \pm 5.4 \times 10^{-3}$
6	$7.60 \pm 4.5 \times 10^{-3}$	$7.57 \pm 5.1 \times 10^{-3}$
7	$5.96 \pm 3.3 \times 10^{-3}$	$5.76 \pm 6.1 \times 10^{-3}$
8	$4.36 \pm 4.5 \times 10^{-3}$	$4.47 \pm 5.5 \times 10^{-3}$
9	$6.96 \pm 3.9 \times 10^{-3}$	$6.79 \pm 4.3 \times 10^{-3}$
10	$1.96 \pm 4.1 \times 10^{-3}$	$2.09 \pm 3.1 \times 10^{-3}$

[a] $X \pm$ S.D.

Table 3
A comparison of previous methods for determining cholesterol

Detection method	Response time (min)	Linear range (mM)	Detection limit (μM)	Lifetime (days)	Known interferents	Ref.
Amperometric	1	2–10 mM for FC	500	60	Ascorbic acid, glucose were major interferents	[42]
Amperometric	1	0.05–0.50	8	25	Not tested	[43]
Potentiometric	16	0.05–3 for TC	10	–	Ascorbic acid, bilirubin and proteins had negligible effects	[44]
Amperometric	<2	0.58–3.68 for FC	60	5	Ascorbic acid, paracetamol, glutathione, uric acid were major interferents	[45]
Amperometric	5	No data for FC	–	–	Oxygen, urea	[46]
Amperometric	1	Not linear for TC	500	1	Not tested	[47]
Spectrophotometric	5	2.6–15.6 for TC	2.6	–	Negligible interference from ascorbic acid, uric acid and haemoglobin	[48]
Spectrophotometric	12	0.13–1.3 for TC	130	–	Testosterone; Vitamin D; progesterone were major interferents. NaCl; $CuSO_4$; creatinine; $NaHCO_3$; albumin; estrogen had practically no effect	[49]
Amperometric	2	0.001–1 for TC	0.012	30	Ascorbic acid, lysine, glucose, lactate, and uric acid had negligible or not effects	Proposed method

FC refers to the fact that the biosensor was used to measure free cholesterol only, while TC means total cholesterol was determined.

4. Conclusions

The usefulness of enzyme biosensor used for the determination of different concentrations of Chol was demonstrated. The proposed method was compared with another biosensor previously developed. Table 3 indicates that the results obtained by the proposed method were better than the results obtained by existing methods. This type of detection shows good promise in biological sensing, particularly in plasma and whole blood samples. Also, this biosensor is able to operate as a fast, selective and sensitive detection unit when is incorporated into a FIA system, and provides a fast and cost effective solution to the realization of quantitative information at extremely high and low levels of Chol concentrations.

Advantages to these biosensors include rapid analysis, reusability, thermal stability, and linearity. The method is highly reproducible and the values correlate well with those obtained with the colorimetric assays.

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