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Screen-printed immunosensor for quantification of human serum IgG antibodies to *Helicobacter pylori*

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Abstract

This paper describes the development of a screen-printed immunosensor for the rapid and sensitive quantification of human serum immunoglobulin G (IgG) antibodies to *Helicobacter pylori*. This microorganism cause peptic ulcers and chronic gastritis, affecting around the 10% of the world population. Antibodies in the serum sample are allowed to react immunologically with the purified *H. pylori* antigens that are immobilized on graphite screen-printed electrodes (GSPE). The bound antibodies are quantified by alkaline phosphatase (AP) enzyme-labeled second antibodies specific to human IgG. *p*-Aminophenyl phosphate (*p*-APP) was converted to *p*-aminophenol (*p*-AP) by AP, and an electroactive product was quantified using Osteryoung square wave voltammetry (OSWV). The electrochemical detection can be done within 1 min and total assay time was 25 min. The calculated detection limits for electrochemical detection and the ELISA procedure are 0.5 and 1.8 U ml^{-1}, respectively. Reproducibility assays were made using repetitive standards of *H. pylori* specific antibody (measured as the activity of the correspondent anti-serum's enzyme conjugated) and the intra- and inter-assay coefficients of variation were below 5%. The electrochemical immunosensor showed higher sensitivity and lower time consumed than the standard spectrophotometric detection ELISA method, demonstrate its potential usefulness for early assessment of human serum immunoglobulin G (IgG) antibodies to *H. pylori*.

Keywords: *Helicobacter pylori*; Alkaline phosphatase; *p*-Aminophenyl phosphate; Screen-printed electrodes; Immunosensor; FIA

1. Introduction

The Gram-negative pathogen *Helicobacter pylori* is specifically adapted to colonize the mucus layer covering the human gastric mucosa. Despite the induction of strong local immune responses in the gastric mucosa of *H. pylori*-infected hosts, the bacterium is capable of establishing chronic infections lasting up to several decades [1] and can cause peptic ulcers and chronic gastritis [2,3]. *H. pylori* infection causes nearly all ulcer diseases that are not brought about by the use of certain medications such as nonsteroidal anti-inflammatory drugs [4]. Evidence linking chronic *H. pylori* infection and gastric cancer has also been demonstrated by epidemiological and pathological studies [5,6].

Within several years of its isolation, *H. pylori* was recognized as the etiologic agent of most gastric and duodenal ulcers and non-autoimmune gastritis, and anti-microbial therapy is now the standard of care for patients with peptic ulcer disease that are positive for *H. pylori* [7].

The disease can be cured by eradication of *H. pylori* through the triple therapies based on a proton pump inhibitor with two antibiotics (clarithromycin and metronidazole or amoxicillin) [8]. In the absence of therapeutic intervention, infection by *H. pylori* lasts for the life of the host, and the robust inflammation and immune response that accompanies infection fails to eradicate the organisms from the gastric epithelium [9].

To prevent the indiscriminate use of multiple antibiotics, an accurate diagnosis for the presence of *H. pylori* infection becomes crucial. Current methods for diagnosing *H. pylori* infection can be divided into invasive and noninvasive (or minimally invasive) [10]. The invasive method uses endoscopy to obtain a biopsy sample of the stomach lining, culturing, and histological examination and urease test. [11,12].

The noninvasive tests include the urea breath test and serological tests. Some noninvasive tests are based on serological procedures that detect immunoglobulin G (IgG)1 against *H. pylori* in human serum. Circulating anti-*H. pylori* IgG antibody

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has proved to be of considerable value in the diagnosis of active infection due to the reliable correlation between the presence of the antibody and gastric mucosal colonization [13,14]. Common serum IgG measurements are carried out using enzyme-linked immunosorbent assay (ELISA) [15]. Although ELISA is a popular assay in clinical examination, the relatively poor limit of detection with absorbance spectrophotometry is one cause of its lack of sensitivity [16].

Heterogeneous enzyme immunoassays, coupled with flow injection (FI) system and electrochemical detection, represent a powerful analytical tool for the determination of low levels of many analytes such as antibodies, hormones, drugs, tumor markers, and viruses [17]. Electrochemical methods typically have the advantage of being highly sensitive, rapid, and inexpensive [18].

In general, enzyme immunoassays combine the specificity of the antigen–antibody reaction with the sensitivity and signal amplification of enzyme-catalyzed reaction. ALP is widely employed for this purpose since its reactions are basically free of interferences, it is highly stable, and it has a high turnover, low cost, and broad substrate specificity [19]. Moreover, its use as a tracer in immunoassays and molecular biology has become popular [20–27].

Screen-printing technique seems to be one of the most promising approaches allowing simple, rapid and inexpensive biosensors production [28]. The biosensors based on screen-printed electrodes have been extensively used for detections of biomolecules, pesticides, antigens, and anions [29].

Electrochemical biosensors based on screen-printed-single-use electrodes are in tune with the requirements of in situ screening devices, since all the equipment needed for the electrochemical analysis is portable. They have all the major performance characteristics of biosensors, among them the minimum sample preparation, the simplicity of the apparatus, the obtaining of fast results, moreover they are cost effective, small and becoming miniaturized with new technologies [30]. Square-wave voltammetry (SWV) is one of the electrochemical techniques more widely applied in quantitative analysis, especially due to its high sensibility, which is a consequence of the rejection of most of the capacitive currents [31,32].

In the presented work, we establish an electrochemical disposable immunosensor with rotation incorporated into an FI analytical system for rapid and sensitive quantification of human serum IgG antibodies to *H. pylori*, based on the use of purified *H. pylori* antigens that are immobilized on graphite screen-printed electrodes (GSPE). Antibodies in the serum sample are allowed to react immunologically with the antigens, and the bound antibodies are quantified by alkaline phosphatase (AP) enzyme-labeled second antibodies specific to human IgG. *p*-Aminophenyl phosphate (*p*-APP) was converted to *p*-aminophenol (*p*-AP) by AP [33,34] and was quantified using Osteryoung square wave voltammetry (OSWV) [35,36].

The response current obtained from the product of enzymatic reaction is proportional to the activity of the enzyme and, consequently, to the amount of antibodies bound to the surface of the immunosensor of interest. These devices may be produced rapidly and inexpensively and electrochemical detection allows simple instrumentation and the possibility of integration of the sensor in a portable device for rapid in-field measurement.

2. Materials and methods

2.1. Reagents and solutions

All reagents used were of analytical reagent grade. AP enzyme-labeled second antibodies specific to human γ-chain was purchased from Sigma Chemical (St. Louis, MO, USA). The 4-nitrophenyl phosphate disodium salt hexahydrate (pNPP) were purchased from Flucka Chemie (Steinheim, Switzerland). GSPE was purchased from EcoBioServices & Researches S.r.l. (Fienze, Italy). All other reagents employed were of analytical grade and used without further purifications. Aqueous solutions were prepared using purified water from a Milli-Q system.

The ELISA test kit for the quantitative determination of *H. pylori*-specific IgG class antibodies was purchased from EQUIPAR Diagnostici (Rome, Italy) and was used in accordance with the manufacturer's instructions [37].

2.2. Flow-through reactor/detector unit

The main body of the cell was made of Plexiglas. Fig. 1 illustrates the design of the flow-through chamber containing the rotating disk and the detector system. The GSPE is on the top of the rotating reactor. The rotating reactor is a disk of Plexiglas into which a miniature magnetic stirring bar has been embedded. Rotation of the lower reactor was effected with a laboratory magnetic stirrer with control of temperature (Metrohm AG, Herisau, Switzerland) and controlled with a variable transformer with an

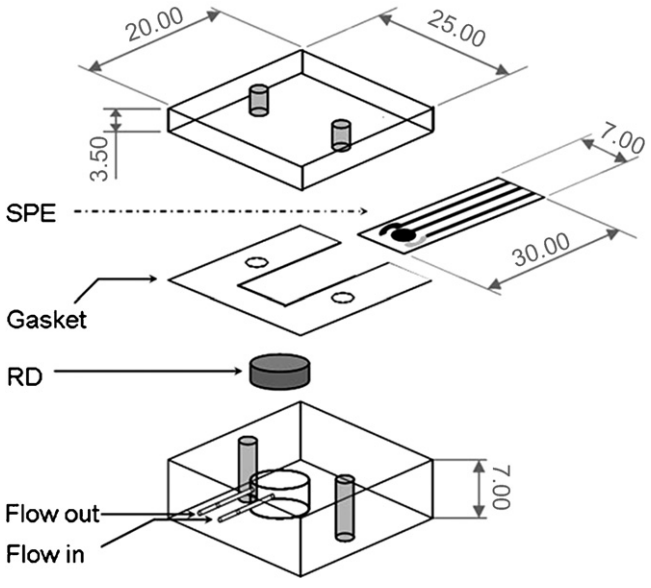

Fig. 1. Schematic representation of components in the bioreactor flow cell. SPE: screen-printed electrode; RD: rotating disk. All measurements are given in millimeters. Gasket: Teflon, thickness 0.3 mm.

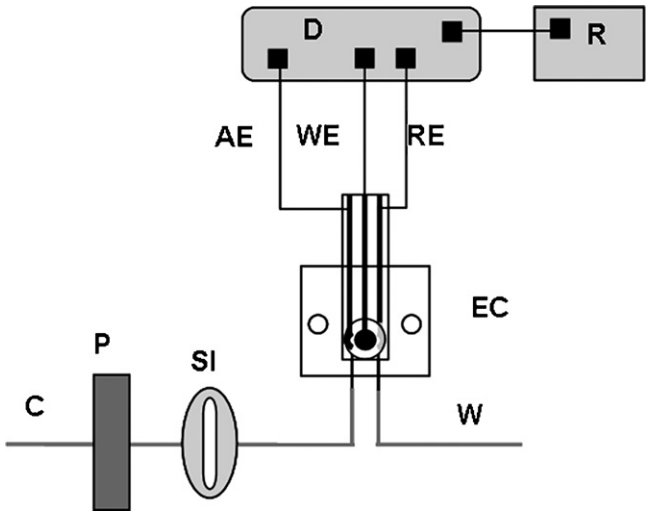

Fig. 2. Block diagram of the continuous-flow system and detection arrangement. P: pump (Gilson Minipuls 3 peristaltic pump, Gilson Electronics Inc., Middleton, WI); C: carrier buffer line; SI: sample injection; W: waste line; EC: cell containing the rotating disk and GSPE; WE: GSPE; RE: pseudo-reference electrode; AE: auxiliary electrode; D: BAS 100B/W electrochemical analyzer (Bioanalytical Systems, West Lafayette, IN, USA); R: recorder.

output between 0 and 250 V and maximum amperage of 7.5 A (Waritrans, Argentina). All solutions and reagents were conditioned to 37 °C before the experiment, using a laboratory water bath Vicking Mason Ii (Vicking SRL, Argentina).

Electrochemical detection was performed using a BAS 100B/W electrochemical analyzer (Bioanalytical Systems, West Lafayette, IN, USA) which was used for cyclic voltammetric analysis and OSWV.

A pump (Wilson Minipuls 3 peristaltic pump, Gilson Electronics, Middleton, WI, USA) was used for pumping, introducing the sample, and stopping the flow Fig. 2 illustrates schematically the components of the single-line continuous-flow setup. The pump tubing was Tygon (Fisher Accu Rated, 1.0 mm i.d., Fisher Scientific, Pittsburgh, PA, USA), and the remaining tubing used was Teflon (1.0 mm i.d. from Cole-Parmer, Chicago, IL, USA).

All pH measurements were made with an Orion Expandable Ion Analyzer (model EA 940, Orion Research, Cambridge, MA, USA) equipped with a glass combination electrode (Orion Research). The absorbancies were detected by Bio-Rad Benchmark microplate readers (Japan) and a Beckman DU 520 general UV/vis spectrophotometer (Fullerton, CA, USA). The *H. pylori* antigens were sonicated by a Sonics Vibra Cell ultrasonic processor (Sonics & Materials, Newtown, CT, USA).

2.3. H. pylori-specific IgG antibody immunoassay

A series of standards that covered the clinically relevant range (0–100 U m1^{-1}) was supplied with the ELISA test kit. A standard curve for the spectrophotometric procedure was produced by following the manufacturer's protocol. Concentrations of *H. pylori*-specific IgG antibody were detected spectrophotometrically by measuring absorbance changes at 450 nm.

2.4. Preparation of the H. pylori antigens

The antigens was prepared from a sonicate *H. pylori* culture strain. The *H. pylori* were grown on blood agar plates at 37 °C for 3 days and then harvested, washed, and resuspended in 0.01 M phosphate-buffered saline (PBS, pH 7.2). This preparation was subjected to sonication. The sonic amplitude level was set at 20, and the machine was operated using four cycles of 60 s regulated alternatively. The sonicated preparation was centrifuged at 1000 × g for 10 min, and the supernatant was stored in the 0.01 M PBS (pH 7.2), at −20 °C between uses.

2.5. Synthesis of p-APP

Synthesis of *p*-APP by catalytic hydrogenation of pNPP was performed using the procedure of reference [18] with the following modifications. In a 100 ml glass hydrogenation vessel, 2.00 g of pNPP was dissolved in 30 ml of 50% ethanol containing 0.11 g of 10% palladium on charcoal catalyst. The hydrogenation reaction was conducted overnight at room temperature at an initial pressure of 1.3 atm. The resultant mixture was filtered on a Butchner funnel to remove the catalyst and the volume of solvent was reduced to 10 ml using a rotary evaporator. The oily residue was diluted to 20 ml with distilled, deionized water and clarified by filtration. Cold ethanol (20 ml, 4 °C) was added to the filtrate and the precipitated product was recovered by filtration, dried under vacuum, and stored at −10 °C. The *p*-APP product was greater than 98% pure as determined by NMR and electrochemical methods.

2.6. Preparation of biosensors

An electrode pretreatment was carried out before each voltammetric experiment in order to oxidize the graphite impurities and to obtain a more hydrophilic surface [38], with the aim of improving the sensitivity and reproducibility of the results. The graphite electrode surface is pretreated applying a potential +1.6 V (versus Ag-SPE) for 120 s and +1.8 V (versus Ag-SPE) for 60 s in 5 ml of 0.25 M acetate buffer, containing 10 mM KCl (pH 4.75), under stirred conditions. Then, the electrodes were washed using 0.01 M PBS, pH 7.2 and stored in the same buffer at 4 °C.

The screen-printed graphite working electrode was modified by a precoating with 5 μl (100 μg ml^{-1} in 0.01 M PBS, pH 7.2) of *H. pylori* antigens and were incubated for 1 h at 37 °C. Following the immobilisation of *H. pylori* antigens by passive adsorption, the electrodes were washed with washing buffer PBS 0.01 M, pH 7.2. They were then blocked by immersion in 200 μl of blocking buffer (3% descremate milk in a 0.01 M PBS, pH 7.2) for 30 min at 37 °C. The immobilized antigens preparation was finally washed with phosphate buffer (pH 7.2) and stored in the same buffer at 5 °C.

2.7. Procedure for electrochemical immunosensor

This method was applied in the determination of IgG antibodies to *H. pylori* in 25 human serum samples. The

serum samples were first diluted 100-fold with 0.01 M PBS (pH 7.2) and then 20 μl was injected into the PBS carrier stream at a flow rate of 0.25 ml min^{-1} and incubated 10 min at 37 °C. The immunoreactor was washed with 0.01 M PBS (pH 7.2) and 20 μl of anti-human IgG–AP conjugate (dilution of 1/2000 in 0.01 M PBS, pH 7.2) was then injected into the 0.01 M PBS (pH 7.2) carrier stream and incubated 10 min at 37 °C. The immunosensor was then washed free of any traces of unbound enzyme conjugate with 0.01 M PBS (pH 7.2).

Diethanolamine buffer (DEA) (100 mM diethanolamine, 50 mM KCl, 1 mM $MgCl_2$, pH 9.6) was used to prepare the *p*-APP solution. Twenty microliter of substrate solution (2.7 mM *p*-APP in a DEA buffer, pH 9.6) was injected into the carrier stream, and allowed to react for 1 min. The rotation was then stopped and the enzymatic product was measured using OSWV (−200 to 300 mV, 30 mV sweep width amplitude, 15 Hz frequency, 10 mV step potential, 10^{-5} A/V, sensitivity samples per point: 256) and the peak current was determined by drawing a tangent line across the base of the peak using the BAS 100W software. For the next assay a new GSPE modified with *H. pylori* antigens was used.

A standard curve for the electrochemical procedure was produced by following our protocol with a series of standards that covered the clinically relevant range (0–100 U ml^{-1}) supplied with the ELISA test kit. Electrochemical measurements were performed at 37 °C and the resulting anodic current was measured.

The stock solution of *p*-APP was prepared freshly before the experiment and stored under the exclusion of light for the duration of the experiment. Blank and zero controls were included in all assays. Blank controls represent electrodes without immobilized antigen and zero controls represent electrodes to which no analyte was exposed. All data points were measured in triplicate ($n = 3$) unless otherwise stated.

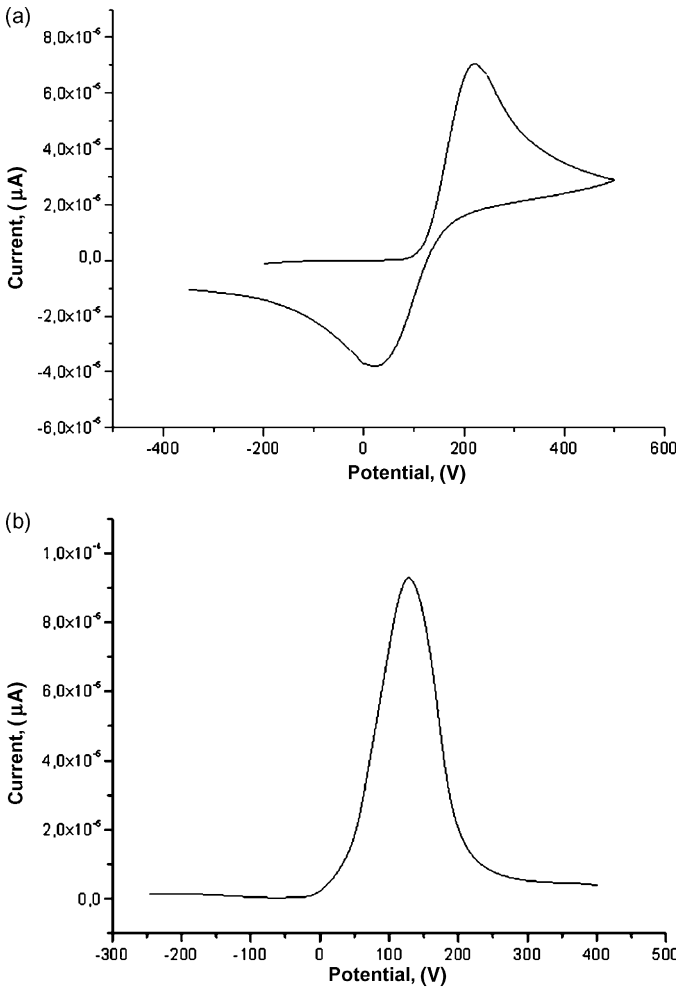

Fig. 3. Electrochemical study of p-AP with the GSPE. (a) Cyclic voltammogram in aqueous solution containing 5.0×10^{-3} mol l^{-1} of *p*-AP in DEA buffer (pH 9.6). Scan rate: 100 mV s^{-1}. (b) A OSWV of 5.0×10^{-3} mol l^{-1} of *p*-AP in DEA buffer (pH 9.6), 30 mV sweep width amplitude, 15 Hz frequency, 10 mV step potential, 10–5 A/V.

3. Results and discussion

3.1. Electrochemical study of p-AP with the GSPE

The electrochemical behaviour of the hydrolysis products (*p*-AP) of the enzyme substrates *p*-APP, was examined by cyclic voltammetry at GSPE. A cyclic voltammetric study of 5.0×10^{-3} mol l^{-1} of *p*-AP in DEA buffer (pH 9.6), was performed by scanning the potential from −300 to 500 mV versus Ag/AgCl. CV showed well-defined anodic and corresponding cathodic peak, which corresponds to the transformation of *p*-AP to *p*-benzoquinoneimine (QI) and vice versa within a quasireversible two-electron process (Fig. 3a).

A OSWV study of 5.0×10^{-3} mol l^{-1} of *p*-AP in DEA buffer (pH 9.6), was performed by scanning the potential from −200 to 300 mV versus Ag/AgCl at, 30 mV sweep width amplitude, 15 Hz frequency, 10 mV step potential, 10^{-5} A/V. The peak current was determined by drawing a tangent line across the base of the peak using the BAS 100W software. Typical responses as represented in Fig. 3b.

3.2. Effect of reactor rotation and continuous-flow/stopped-flow operation

To optimize the proposed method is necessary to have an understanding of the effect of the parameters governing the system. It has been shown that the theoretical framework developed for static ELISA system cannot be applied to describe the kinetics of antibody–antigen interactions occurring in a continuos flow immunoassay [39]. Several parameters differ significantly. First, buffer flow reduces the limitations of diffusion as observed in static ELISA systems. Second, the association rate of an antibody with an immobilized antigen has been reported to decrease with increasing flow rates [40]. Furthermore, the surface density of immobilized antibodies/antigens in the flow immunoassay is at least three orders of magnitude lower than in static ELISA systems, but a high sensitivity can be attained by a rotating bioreactor and continuous-flow/stopped-flow/continuous-flow processing [41].

The proposed method manifolds follow the ELISA principles, but instead of using a microtiter plate, the reagents and

washing buffers were pumped consecutively through the reactor containing immobilized antigen, on graphite screen-printed electrodes (GSPE), coupled to electrochemically detection for the determination of human antibody. AP enzyme-labeled second antibodies specific to human γ-chain was used as conjugate and the product of the enzymatic indicator reaction was measured using OSWV.

The implementation of continuous-flow/stopped-flow programming and the location of two facing independent reactors (Fig. 1) permits: (a) utilization of relatively low immunoreactants 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 [42], because the cell works as a mixing chamber by facilitating the arrival of immunoreactants at the specific antigens, the arrival of enzymatic substrate at the active sites and the release of products from the same sites. The net result is high values of current. The main advantages of this system are its simplicity, and the easy with which it can be applied to the determination of specific IgG antibodies to *H. pylori* in serum samples.

The effect of the rotation velocity was evaluated in a range of 60–300 rpm. A significant increase of electric signal was observed between 60 and 200 rpm. Insignificant differences were obtained for greater rotation velocities. For convenience a rotation velocity of 200 rpm was used to evaluate other parameters (Fig. 4).

If the rotating disk in the cell is devoid of rotation, the response is lower because diffusional reactions are too slow to be observed in the time scale of electrochemical analysis. If a rotation of 200 rpm is imposed on the rotating disk at the bottom of the cell, the signal is dramatically enlarged (Fig. 5).

The response current obtained from the oxidation of *p*-AP in buffer DEA is proportional to the activity of the enzyme conjugated and consequently, to the amount of specific antibodies of

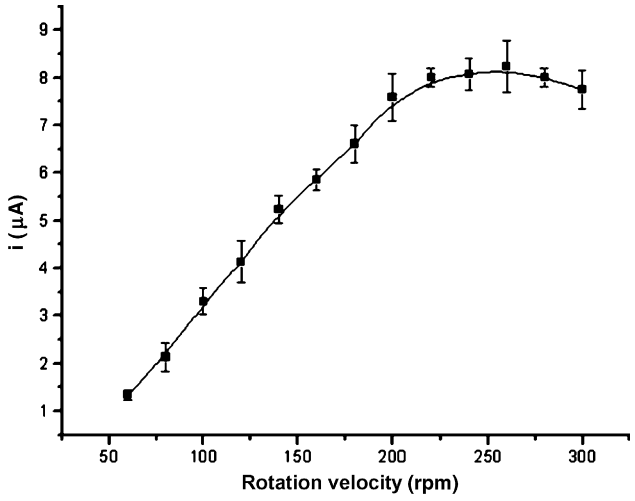

Fig. 4. Effect of the rotation velocity. Each value of initial rate based on triplicate of six determinations. Flow rate 0.25 ml min^{-1}. 2.7 mM *p*-APP in a DEA buffer, pH 9.6. The ppflow was stopped and allowed to react for 1 min. The rotation was then stopped and the enzymatic product was measured using OSWV.

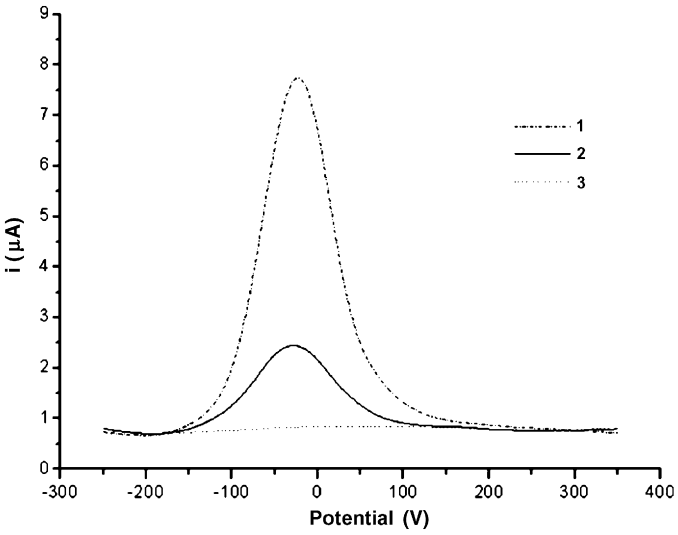

Fig. 5. Effect of reactor's rotation. (1) Stopped flow with rotation, (2) stopped flow without rotation and (3) blank. Flow rate 0.25 ml min^{-1}, 2.7 mM *p*-APP in a DEA buffer, pH 9.6. The flow was stopped and allowed to react for 1 min. The rotation was then stopped and the enzymatic product was measured using OSWV.

serum samples bound to the rotating disk with *H. pylori* antigens immobilized.

As noted, rotation is expected to decrease the values of the apparent Michaelis-Menten constant K'_M, since the catalytic efficiency is increased. K'_M which differ substantially from that measured in homogeneous solution and 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 [43].

3.3. Effect of cell volume and sample size

Depending on the volume of the cell the overall process becomes controlled by diffusion (large volumes) or by the chemical kinetics of the immune and enzyme-catalyzed reactions (small volumes). The cell volume was changed from 20 to 100 μl. The rate of response, as expected, decreased linearly with an increase in cell volume, due to the dilution effect favoured by rotation, and the fact that the measured current is directly proportional to bulk concentration. The smallest cell volume of 20 μl was adopted for further studies.

The sample size was studied in the range 5–50 μl. Sensitivity is almost tripled in the range between 5 and 20 μl (Fig. 6). Insignificant differences were obtained for greater sample size. A sample size of 20 μl was used to evaluate other parameters.

3.4. Optimum conditions for the determination of the enzymatic products

The rate of enzymatic response under stopped-flow conditions was studied in the pH range 7–11 and show a maximum value of activity at pH 9.6. The pH value used was 9.6 in DEA buffer. The effect of varying *p*-APP concentration from

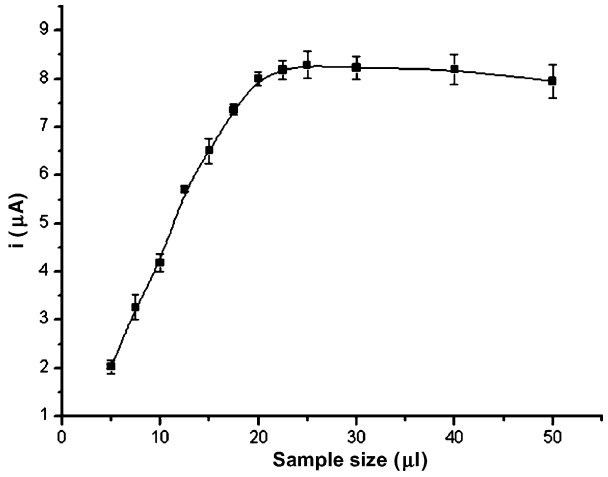

Fig. 6. Effect of sample size. The initial rate was measured under stopped-flow conditions. Each value of i (μA) based on five determinations. Flow rate, 0.25 ml min^{-1}; the reactor rotation velocity was 200 rpm. The flow was stopped and allowed to react for 1 min. The rotation was then stopped and the enzymatic product was measured using OSWV.

0.1 to 5 mM on the immunosensor response was evaluated. The optimum *p*-APP concentration found was 2.7 mM. That concentration was then used.

An additional parameter which would affect the assay was the incubation temperature. Different incubation temperature was reported in literature ranging from 25 to 37 °C [44,45]. As well known, an optimal temperature of immunoreaction would be 37 °C.

3.5. Quantitative test for the detection of H. pylori specific IgG antibody

Under the selected conditions described above, the electrochemical response of the enzymatic product is proportional to the concentration of *H. pylori* specific IgG antibody in the serum.

A linear calibration curve for the detection of *H. pylori* specific IgG antibody in serum was produced over the range of 0–100 U ml^{-1}. The linear regression equation was $i = 0.086 + 0.078$CHp, with the linear relation coefficient $r = 0.998$. The coefficient of variation (CV) for the determination of 20 U ml^{-1} *H. pylori* specific antibody was 2.7% (six replicates). The ELISA procedure was also carried out as described, absorbance changes were plotted against the corresponding *H. pylori* specific IgG antibody concentration and a calibration curve was constructed. The linear regression equation was $A = 0.031 + 0.029$CHp, with the linear relation coefficient $r = 0.996$, the CV for the determination of 20 U ml^{-1} *H. pylori* specific antibodies was 4.4% (six replicates).

Taking the detection limit to be the concentration that gives a signal three times the standard deviation (S.D.) of the blank; for electrochemical detection and ELISA procedure was 0.5 and 1.8 U ml^{-1}, respectively. This result shows that electrochemical detection was more sensitive than spectrophotometric method.

The sensitivity (S) is defined as the slope of the regression line signal versus concentration, S for electrochemical detection and ELISA procedure was 0.078 μA/(U ml^{-1}) and 0.029 Abs/(U ml^{-1}), respectively.

Table 1
Within-assay precision (five measurements in the same run for each control serum) and between-assay precision (five measurements for each control serum, repeated for 3 consecutive days)

Control sera[a] (U ml^{-1})	Within-assay		Between-assay	
	Mean	CV%	Mean	CV%
20	20.22	22.1	21.22	2.98
50	50.08	1.96	49.04	4.12
100	99.24	2.68	98.93	3.20

[a] U ml^{-1} *H. pylori* specific antibodies.

The precision of the electrochemical assay was checked with control serum at 20, 50 and 100 U ml^{-1} *H. pylori* specific antibody concentrations. The within-assay precision was tested with five measurements in the same run for each serum. These series of analyses were repeated for three consecutive days in order to estimate the between-assay precision. The results obtained are presented in Table 1. The *H. pylori* assay showed good precision; the CV within-assay values were below 3% and the between-assay values were below 5%.

The accuracy was tested with dilution and recovery tests. A dilution test was performed with 100 U ml^{-1} *H. pylori* specific antibodies control sera with 0.01 M PBS, pH 7.2 (Fig. 7).

Reproducibility assays were made using a repetitive standard ($n = 6$) of 20 U ml^{-1} *H. pylori* specific antibody; the percentage standard error was less than 3% (Table 2).

3.6. Correlations with ELISA assay procedure

The electrochemical system was compared with a commercial spectrophotometric system for the quantification of *H. pylori* specific antibodies in 25 serum samples. The slopes obtained were reasonably close to 1, indicating a good correspondence between the two methods (Fig. 8). Compared with the ELISA, our method shows large enhancement in sensitiv-

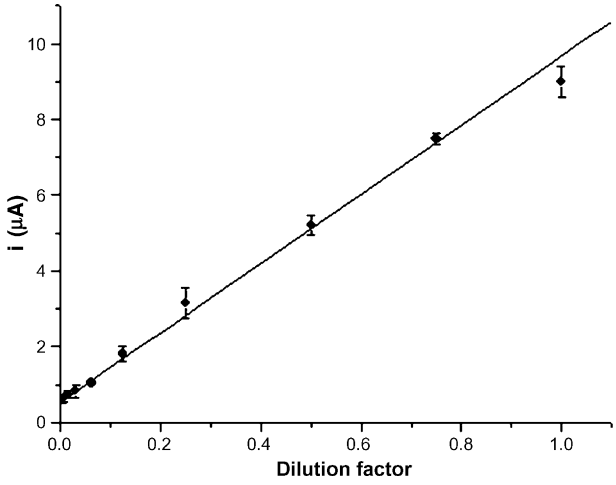

Fig. 7. Dilution test results for 100 U ml^{-1} *H. pylori*-specific antibodies. Each value of i (μA) is based on five determinations.

Table 2
Reproducibility assays using repetitive standards ($n = 6$) of 20 U ml^{-1} *H. pylori* specific antibody

Standards of 20 U ml^{-1} *H. pylori* specific antibody	Proposed method (U ml^{-1})	ELISA (U ml^{-1})
1	20.18	21.43
2	19.87	19.37
3	20.42	20.47
4	19.78	19.74
5	19.83	20.92
6	20.24	21.86
[a] $X \pm$ S.D.	20.06 ± 0.26	20.63 ± 0.96

[a] U ml^{-1}, mean ± S.D., standard deviation.

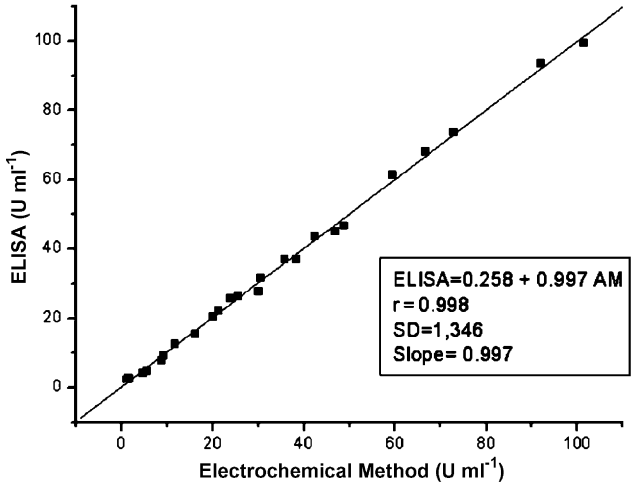

Fig. 8. Correlation between proposed method and commercial photometric assays.

ity. These results suggest that the detectable concentration of *H. pylori* specific antibodies in this system has been already at the levels of clinical analysis, and the sensitivity has reached to the levels to meet the determination of *H. pylori* specific antibodies in serum even in light infected degree serum.

4. Conclusions

In this work, a disposable electrochemical immunosensor coupled with flow injection (FI) system for rapid sensitive and selective quantification of specific antibodies against *H. pylori* in human serum sample was developed using a GSPE system as transducer for electrochemical detection.

The attachment of purified *H. pylori* antigens on the electrode surface is performed in a simple way by physical adsorption in contrast to other methodologies reported in the literature where a covalent bound is necessary, and the integration of the disposable immunosensor and the flow cell meets the demand of facilitating the electrochemical immunoassay process. The overall assay time (25 min) was shorter than the time reported for ELISA commercially test kits (160 min), this may be possible without reduced selectivity, being these an important advantage. Also minimizes the waste of expensive antigens and other reagents; shows physical and chemical stability, low background current, wide working potential range, and accuracy.

In conclusion we took advantage of the simplicity of the ELISA system to construct a immunosensor that was capable of measuring the same levels of specific antibodies against *H. pylori* in human serum sample as detected by the conventional methods while having the advantages of low detection limit, speed and simplicity. Analytical results of clinical samples show the developed immunoassay has a promising alternative approach for detecting specific antibodies against *H. pylori* in human serum sample in the clinical diagnosis.

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