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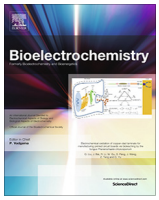

Unraveling autoimmune and neurodegenerative diseases by amperometric serological detection of antibodies against aquaporin-4

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ABSTRACT

This work reports the first electroanalytical bioplatform to date for the determination of antibodies against aquaporin-4 (AQP4-Abs), whose serum level is considered as relevant biomarker for certain autoimmune diseases. The bioplatform relies on the use of magnetic microparticles modified with the biotinylated protein for the capture of specific antibodies. The captured IgGs are enzymatically labelled with a secondary antibody conjugated to the horseradish peroxidase (HRP) enzyme. Amperometric transduction is performed using the H_2O_2/hydroquinone (HQ) system, which results in a cathodic current variation directly proportional to the concentration of the target antibodies. The evaluation of the analytical and operational characteristics of the developed bioplatform shows that it is competitive in terms of sensitivity with the only biosensor reported to date as well as with the commercially available ELISA kits. The achieved limit of detection value is 8.8 pg mL^{-1}. In addition, compared to ELISA kits, the developed bioplatform is advantageous in terms of cost and point of care operation ability. The bioplatform was applied to the analysis of control serum samples with known AQP4-Abs contents as well as of sera from healthy individuals and patients diagnosed with Systemic Lupus Erythematosus (SLE) and Alzheimer (AD) diseases, providing results in agreement with the ELISA methodology.

1. Introduction

In an allergic reaction, a viral or bacterial infection, and in many other diseases, when certain self-antigens (nucleic acids, proteins, nuclear molecules, receptors, or other functional cell components) are altered due to overexpression or some aberrant modification, the immune system reacts by producing self-reactive antibodies against them, called natural antibodies or autoantibodies.

The level of autoantibodies in serum (mostly immunoglobulins G, IgGs) plays a key role in the diagnosis and classification of a large number of disorders and illnesses. Autoantibody levels can be used for the early detection of the disease, since several longitudinal cohort studies showed that patients can carry autoantibodies years before the manifestation of clinical symptoms [1,2], thus having a strong predictive value. Moreover, autoantibody concentration can be employed for monitoring the evolution of the disease, since once cure occurred, the production of autoantibodies should progressively decrease to the basal level of healthy individuals. It is important to note that autoantibodies are more stable than their specific antigens in blood, and they are indicative of very low concentrations of the target antigens due to amplification by the immune system.

The important advantages as biomarkers of autoantibodies have driven the search for new specific autoantigens to aid in the minimally invasive diagnosis and monitoring of prevalent chronic diseases.

In the case of autoimmune diseases such as Graves' disease, systemic lupus erythematosus (SLE), multiple sclerosis (MS), rheumatoid arthritis (RA), or neurological disease disorders (NDDs) that are thought to be linked with autoimmune diseases, including a heterogeneous group of chronic disorders like Alzheimer's disease (AD), Parkinson's disease (PD), or dementia with Lewy bodies (DLB), protein misfolding, aggregation and dysregulation of specific autoantigens and corresponding self-antibodies are demonstrated key factors for disease development and evolution [3].

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Autoantibodies against nuclear antigens, cyclic citrullinated peptide, thrombin, rheumatoid factor, thyrotropin receptor and aquaporin-4 have been found to be associated with autoimmune diseases.

Aquaporin-4 protein (AQP4) is a protein belonging to the aquaporin family (with 13 members, AQP0-12), expressed by astrocytes as a long (M−1) or short (M−23) isoform [4]. AQP4 is the most expressed AQP in the mammalian brain and is considered of crucial importance in facilitating the removal of waste products from the brain [5,6]. AQP4 has been proposed as the main autoimmune target of neuromyelitis optica (NMO), also known as Devic syndrome, an immune-mediated neurological disease affecting the spinal cord and optic nerves [7]. Autoantibodies against AQP4 (called NMO-IgGs or AQP4-Abs) are involved in the immunopathogenesis of NMO and are highly specific serum marker to differentially diagnose NMO and MS [5,8–19]. Interestingly, an AQP4-Abs seropositive status has also been associated with other autoimmune diseases such as autoimmune thyroiditis and SLE [20]. Indeed, recent works indicate that some patients with juvenile SLE develop AQP4-Abs and may be at risk for developing a neurologic clinical phenotype [21].

All these precedents show the interest of AQP4-Abs determination as a powerful tool for the differential diagnosis of NMO and as a biomarker in a variety of autoimmune diseases and NDDs. Enzyme-linked immunosorbent assay (ELISA) kits based on different immunoassay formats are commercially available (detailed information and characteristics in **Table S1** in the Supporting Information) for determining AQP4-Abs. However, these methods require specialized instrumentation which restricts their application to centralized environments.

Within this context, electrochemical biosensors are an interesting alternative due to their affordable cost, simplicity of use, and compatibility with point-of-care (POC) operation. In fact electrochemical biosensors have recently demonstrated to be competitive tools for the identification, validation and determination of autoantibodies against tumor-associated antigens in serum [22] or in exosomes released from tumor cells or tissues [23] using HaloTag fusion proteins, as well as of relevance in Alzheimer's disease using phage-displayed or aberrant peptides as bioreceptors [24]. However, to our knowledge only one biosensor appears in the literature for the determination of AQP4-Abs. It involves the use of a carbon nanotube field-effect transistor (CNT-FET) functionalized with AQP4 extracellular loop peptides and achieves a LOD value of 1 ng mL^{-1} [25]. However, the biosensor was only applied to the antibodies determination in a supplemented commercial human serum sample, and not in real samples from patients.

Here we report the first magnetic microbeads (MBs)-based bioplatform for the determination of AQP4-Abs. The developed strategy involves the use of MBs functionalized with streptavidin (Strept-MBs) and modified with biotinylated-AQP4 (b-AQP4) for the selective capture of immunoglobulins against this protein. The detection of IgG attached to the AQP4-b-Strep-MBs is performed by labelling with a secondary antibody conjugated to the HRP enzyme (HRP-antihIgG) and amperometry after capturing the resulting magnetic bioconjugates (HRP-antihIgG-Abs-AQP4-b-Strept-MBs) on the working electrode of a screen-printed carbon electrode (SPCE). In the presence of the H_2O_2/HQ system, the measured cathodic current variation is directly proportional to the concentration of AQP4-Abs. Under the selected experimental conditions, the developed bioplatform was employed for the determination of AQP4-Abs in serum controls with known concentration of target Abs and in serum samples from patients diagnosed with autoimmune diseases (SLE) and NDDs (AD).

2. Experimental

2.1. Reagents and solutions

Biotin-AQP4 conjugate (b-AQP4) and AQP4-Abs standard (100 ng mL^{-1}), sample diluent, HRP-avidin diluent and wash buffer were components of the human AQP4 Abs ELISA kit from Cusabio (Cat. No. CSB-E13568h). HRP-labelled goat anti-human IgG-HRP (HRP-antihIgG, 1 mg mL^{-1}) was from Abcam (Cat. No. ab97225). Streptavidin-modified magnetic microparticles (Strep-MBs, 2.8 μm Ø, 10 mg mL^{-1}, Ref: 11205D) were purchased from Invitrogen-Thermo Fisher™. Sodium chloride, potassium chloride, sodium di-hydrogen phosphate, di-sodium hydrogen phosphate and Tris-hydroxymethyl-aminomethane-HCl (Tris-HCl) were purchased from Scharlab. Hydroquinone (HQ), and hydrogen peroxide (H_2O_2, 30% w/v) were purchased from Sigma-Aldrich. Bovine serum albumin (BSA 100%, Type VH, Cat. No. 90604–29-8) was from Gerbu Biotechnik, GmbH. Human hemoglobin (HB, Cat. No. H7379), albumin from human serum (HSA, Cat. No. A1653), IgG from human serum (hIgG, Ref: I2511) all from Sigma-Aldrich, and anti-dsDNA Abs standard (Cat. No. DEIA 1681) from Creative Diagnostics, were used for selectivity studies.

Positive and negative serum control samples were provided in the AQP4-Abs ELISA Version 2 Kit from RSR Limited.

Water purified by the Milli-Q purification system (18.2 MΩ cm) was used for the preparation of all solutions. The used buffers included: B&W buffer pH 7.5 (0.01 mol L^{-1} Tris-HCl pH 7.5 containing 1 mmol L^{-1} EDTA and 2 mmol L^{-1} NaCl), 0.01 mol L^{-1} phosphate buffer saline solution (PBS) pH 7.5 and 0.05 mol L^{-1} phosphate buffer (PB) solution pH 6.0.

The solutions used to perform the amperometric detection: 0.1 mol L^{-1} H_2O_2 and 0.1 mol L^{-1} HQ, were freshly prepared in phosphate buffer solution pH 6.0.

2.2. Apparatus and electrodes

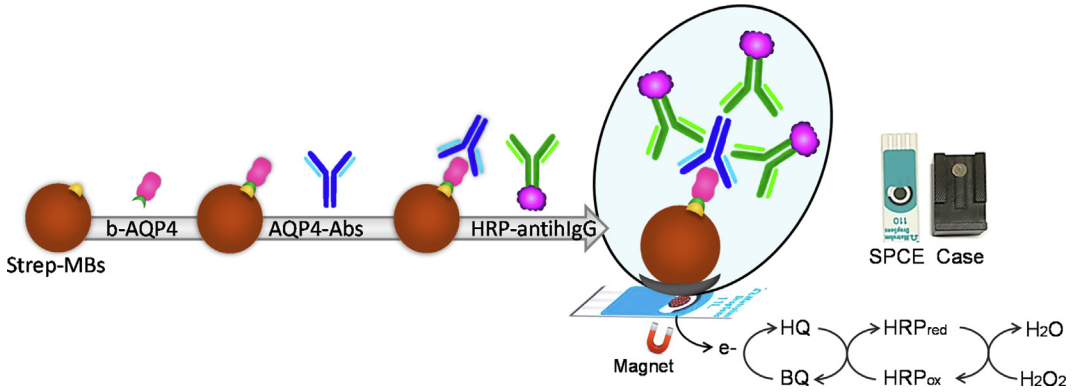
A magnetic concentrator DynaMag-2 (Cat. No. 12321D, Dynabeads®, Invitrogen-Thermofisher Scientific), a Bunsen AGT-9 Vortex, a Thermo-Shaker MT100 (Universal Labortechnik), a P-Selecta (Cencom) centrifuge, a Crison model Basic 20 + pH-meter, an Elmasonic S-60 (Elma) ultrasonic bath and a magnetic stirrer MS01 (ElMI, Ltd.) were employed.

All the amperometric measurements were made at room temperature using a model CHI812B (CH Instruments, Inc.) potentiostat controlled by the CHI812B software. Screen-printed carbon electrodes (SPCEs, DRP110, 4-mm Ø) and a specific cable connector (DRP-CAC) from Metrohm-DropSens were used. The measurements were performed in stirring solutions using 10 mL glass electrochemical cells from Pobel, where the SPCEs placed on a homemade polymethylmethacrylate (PMMA) casing with a neodymium magnet (AIMAN GZ) embedded were immersed.

ELISA absorbance readings were made in a Sunrise™ Tecan microplate reader with the Magellan V 7.1 software.

2.3. Preparation of the magnetic immunoconjugates

The protocol for the preparation of immunoconjugates on the MBs (scheme shown in Scheme 1), including washing and incubation steps, was performed in 1.5 mL microcentrifuge tubes which were placed in the magnetic concentrator for 2 min before removing the supernatant. A 5-μL aliquot of the Strep-MBs suspension was washed twice with 50 μL of B&W buffer and modified with b-AQP4 by incubation for 30 min (37 °C, 950 rpm) with 25 μL of the b-AQP4 solution, 100-fold diluted in B&W buffer. After washing twice with 0.01 mol L^{-1} PBS solution, AQP4-b-Strep-MBs were



Scheme 1. Schematic diagram of the bioconjugate preparation and amperometric transduction steps involved in the determination of AQP4-Abs with the developed bioplatform.

incubated for 60 min (37 °C, 950 rpm) with 25 μL of the AQP4-Abs standard or the serum sample (in sample diluent). Upon washing twice with PBS solution, the Abs-AQP4-b-Strep-MBs were enzymatically labelled by incubation with 25 μL of 2,500-fold diluted (in HRP-avidin diluent) HRP-antihIgG solution for 30 min (37 °C, 950 rpm). The resulting magnetic bioconjugates were washed twice with PBS solution and resuspended in 50 μL of 0.05 mol L^{-1} PB solution (pH 6.0) to carry out the amperometric transduction.

2.4. Amperometric detection

Once the SPCE was placed on the homemade PMMA casing with the embedded neodymium magnet, the 50 μL modified MBs suspension were cast onto the working electrode surface. Then, the casing-electrode assembly was connected through the specific cable to the potentiostat and was immersed in the electrochemical cell containing 10 mL of 0.05 mol L^{-1} PB (pH 6.0) and 100 μL of freshly prepared 0.1 mol L^{-1} HQ solution. A constant potential of −0.20 V vs. Ag pseudo-reference electrode was applied under stirring conditions. Upon stabilization of the background current (approximately 100 s), 50 μL 0.1 mol L^{-1} H_2O_2 enzymatic substrate (freshly prepared in PB, pH 6.0) were added to the cell, and the cathodic current variation occurring by the enzymatic reduction of H_2O_2 mediated by HQ was recorded until the steady state was reached. Amperometric signals were calculated as the difference between such steady state currents and the background currents. The given values are the mean value of three replicates and the error bars were estimated as three times the standard deviation of each set of replicates (α = 0.05).

2.5. Analysis of serum samples

The developed immunosensing bioplatform was used to analyze serum samples from control subjects. Sera from AD patients were provided by Institute of Health Carlos III, after approval of the Institutional Ethical Review Boards and with the written informed consent of all participating individuals. Sera from SLE patients were purchased from Central BioHub. All the experiments were performed accomplishing all the ethical issues and relevant guidelines and regulations of the involved institutions. The samples were stored at −80 °C until use. Once the absence of matrix effect was verified for samples diluted 2,500 times or more, the determination was made by simple interpolation of the signals obtained for the 2,500-diluted samples into the calibration graph constructed with the AQP4-Abs standards.

3. Results and discussion

Scheme 1 illustrates the employed methodology involving an immunoassay strategy assisted by the use of Strep-MBs functionalized with b-AQP4 for the selective capture of AQP4-Abs. The target Abs were detected, prior enzymatic labelling with a secondary antibody and magnetic capture on the surface of the SPCE working electrodes, by amperometry using H_2O_2 as enzymatic substrate and HQ as redox mediator. The measured cathodic current variation, attributed to HQ-mediated enzymatic reduction of the substrate, was directly proportional to the concentration of AQP4-Abs.

3.1. Optimization of the experimental variables

The influence of the following key variables on the bioplatform performance was evaluated: b-AQP4 dilution (a) and incubation time (b) for immobilization on Strep-MBs, AQP4-Abs incubation time for their capture on AQP4-b-Strep-MBs (d), HRP-antihIgG dilution (e) and incubation time (f) for enzymatic labelling of the captured AQP4-Abs, as well as the number of incubation steps used for capturing and enzymatic labelling of AQP4-Abs on AQP4-b-Strep-MBs (c). The amperometric responses provided by the immunoplatforms in the absence (blank, B) and in the presence of 2.5 ng mL^{-1} AQP4-Abs standards (signal, S) and the S/B ratio were compared (Fig. 1). Moreover, the experimental variables involved in the amperometric transduction (detection potential, pH of the supporting electrolyte and H_2O_2 and HQ concentrations) were those optimized in previous works [26–28].

As can be deduced, larger S/B ratios were obtained when Strep-MBs were modified by incubation in a 100 times diluted b-APQ4 solution for 30 min (Fig. 1**a and 1b**, respectively). The lower S values obtained with larger b-APQ4 concentrations (Fig. 1**a**) can be attributed to steric hindrance when large antigen concentrations are immobilized on Strep-MBs [29]. It is important to highlight the lack of discrimination (S/B ∼ 1) found in the absence of b-AQP4, in agreement with the rationale of the methodology. Key to the simplicity and the time lasted by the assay is to evaluate whether capture and labelling of the target AQP4-Abs can be performed in a single step or should be done in different steps. Fig. 1**c** shows as the S/B ratio was notably better when the capture of the target Abs and their enzymatic labelling was carried out in independent steps, which can be attributed to the less efficient capture of the target Abs when they are labelled with the secondary antibody. Therefore, the 2-steps protocol was selected for further work. Fig. 1**d** shows that a 60 min incubation time is suitable for an efficient capture of AQP4-Abs, with worse S/B ratios for longer incubation possibly due to aggregation phenomena. Fur-

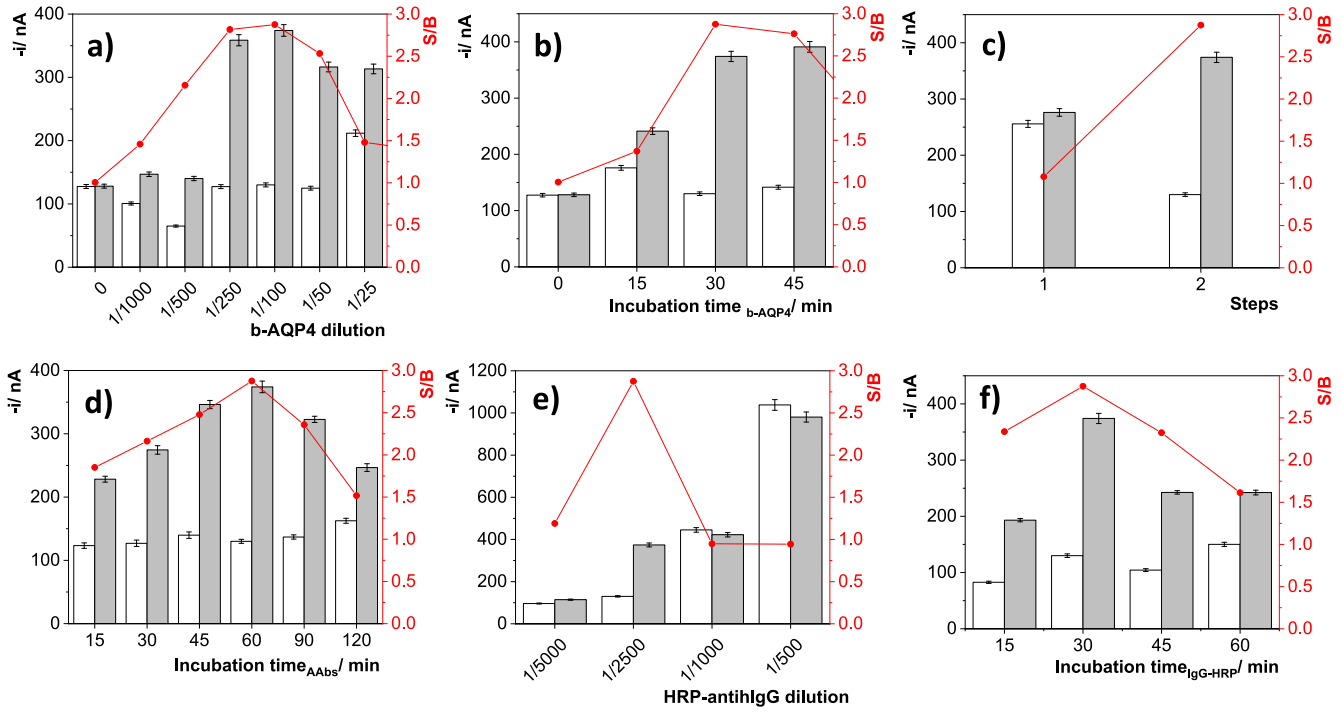


Fig. 1. Dependence of the amperometric responses provided by the developed bioplatforms in the absence (blank, B, white bars) and in the presence of 2.5 ng mL^{-1} AQP4-Abs standards (signal, S, grey bars) as well as the corresponding S/B ratio with the b-AQP4 dilution (a) and incubation time (b) for their immobilization on Strep-MBs, number of incubation steps: (1) capture and labelling of the target AQP4-Abs in a single step or (2) in two successive independent incubation steps (c), AQP4-Abs incubation time for their capture on AQP4-b-Strep-MBs (d), HRP-antihIgG dilution (e) and incubation time (f) for enzymatic labelling of the captured AQP4-Abs.

thermore, larger S/B ratios were found for enzymatic labelling of the captured AQP4-Abs on AQP4-b-Strep-MBs by incubation with a 1/2,500 diluted HRP-antihIgG solution (Fig. 1**e**) for 30 min (Fig. 1**f**). The optimized variables and the selected values are summarized in Table 1. Accordingly, the determination of AQP4-Abs starting from the prepared AQP4-b-Strep-MBs requires 2 incubation steps and 90 min.

3.2. Analytical and operational characteristics of the bioplatform

The analytical and operational characteristics of the developed bioplatform for the amperometric determination of AQP4-Abs standards were evaluated. The calibration graph displayed in Fig. 2 exhibits a linear dependence of the cathodic current variation with the logarithm of the AQP4-Abs standard concentration over the 0.03 to 2.5 ng mL^{-1} range (r^2 = 0.993; slope = (96 ± 4) nA^{-1}; intercept = (332 ± 4) nA). Concentrations larger than 2.5 ng mL^{-1} provoked a drastic decrease in the amperometric response attributed to the high-dose Hook effect due to the saturation of the b-AQP4 and the HRP-antihIgG by the high analyte concentration [30].

The values of the limits of detection and quantification, were estimated according to the 3 × s_b/m and 10 × s_b/m criteria, where s_b was the standard deviation of 10 amperometric measurements in the absence of AQP4-Abs, and m is the slope of the calibration graph. The calculated values were 8.8 and 29.2 pg mL^{-1}, respectively.

The amperometric responses for 0.5 ng mL^{-1} AQP4-Abs standard obtained with 10 bioplatforms prepared from different HRP-antihIgG-Abs-AQP4-b-Strep-MBs batches resulted in a relative standard deviation (RSD) value of 2.4 %, which confirmed the reproducibility of the protocols used for the implementation of the bioassay format on the MBs and for the amperometric transduction at the SPCEs.

The comparison of the amperometric responses for 0 and 2.5 ng mL^{-1} AQP4-Abs obtained with bioplatforms prepared from AQP4-b-Strep-MBs stored in filtered PBS at 4 °C (**Figure S1** in the Supporting Information) allows deducing that the bioconjugates were stable for 21 days. Therefore, during this period it is feasible to prepare the bioplatforms from stored AQP4-b-Strep-MBs, thus allowing the determination can be accomplished using two incubation protocols and lasting only 90 min.

The lack of similar strategies reported in the literature limits the comparison of the developed bioplatform analytical performance to that claimed for the commercial ELISA kits summarized in **Table S1** (in the Supporting Information). Such comparison allows us to deduce that the bioplatform is competitive in terms of sensitivity (LOD 44 times lower than the most sensitive Cusabio ELISA kit which involves the same immunoreagents that the developed bioplatform), assay time (90 min starting from AQP4-b-Strep-MBs vs. 1 h 20 min-4 h 20 min) and affordable and easy to use instrumentation. In addition, the developed bioplatform achieves a considerably smaller LOD than the only reported biosensor for the determination of AQP4-Abs (8.8 pg mL^{-1} vs. 1 ng mL^{-1} [25]). This makes the bioplatform particularly suitable for analysing and evaluating the potential of these antibodies as reliable markers in diseases where their concentration is small.

Table 1
Optimization of the experimental variables for the preparation of the HRP-antihIgG-Abs-AQP4-b-Strep-MBs bioconjugates used for the amperometric determination of AQP4-Abs.

Variable	Tested range	Selected value
b-AQP4 dilution	1/1,000–1/10	1/100
b-AQP4 incubation time, min	15–60	30
AQP4-Abs incubation time, min	15–120	60
HRP-antihIgG dilution	1/5,000–1/500	1/2,500
HRP-antihIgG incubation time, min	15–60	30
Incubation steps	1–2	2

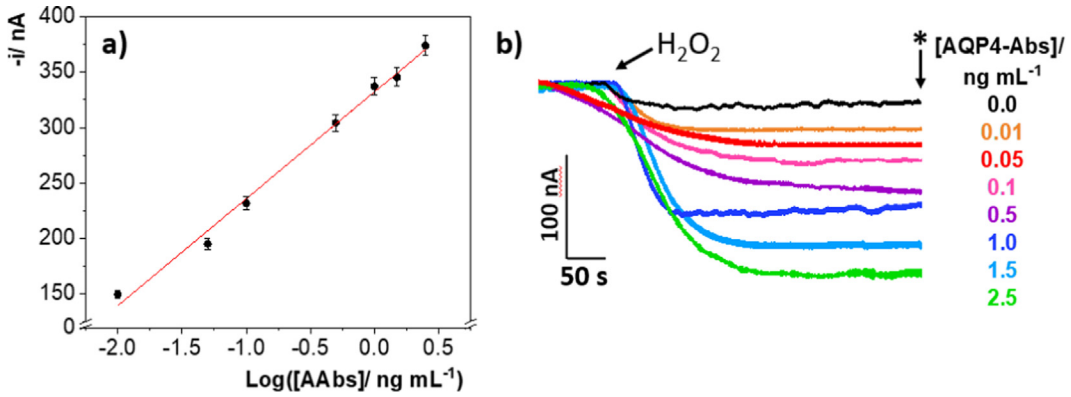


Fig. 2. Calibration plot constructed with the developed bioplatform for the amperometric determination of AQP4-Abs standards (a). Real amperograms recorded (b). *: point where the current was measured.

3.3. Selectivity

In order to test the bioplatform suitability for the determination of AQP4-Abs in serum, we evaluated the effect of the presence of other major serum components (hIgG, HSA) or that may be present (HB and dsDNA-Abs) on the amperometric responses provided by the bioplatforms for 0 and 2.5 ng mL^{-1} AQP4-Abs in the absence and in the presence of such potential interfering compounds. Fig. 3 shows as significantly larger currents were only obtained in the presence of hIgG, which can be attributed to the non-specific adsorption of hIgG on the Strep-MBs. In fact, a similar behaviour was previously observed for a bioplatform developed for the determination of antibodies against dsDNA [31]. However, it is important to note that the discrimination between the presence and absence of AQP4-Abs (S/B ratio) was not significantly affected. In addition, the high sensitivity achieved with the developed bioplatform allows the use of highly diluted serum samples (1/2,500), which led to no significant differences in the S/B ratio for such serum dilution level (Fig. 3).

3.4. Determination in control serum samples and in sera from patients diagnosed with autoimmune and NDDs

The accuracy of the developed bioplatforms was tested by analysing one negative and two positive (I and II) control serum samples provided in the AQP4-Abs ELISA Version 2 Kit from RSR Limited. The measurements obtained with the bioplatform for the undiluted controls were interpolated into a calibration constructed with the standards (1.5, 5, 20, 40, 80 U mL^{-1} AQP4-Abs) provided in the kit. The results obtained (α = 0.05, n = 3) are given in Table 2 and confirmed an excellent accuracy of the results provided by the developed bioplatform.

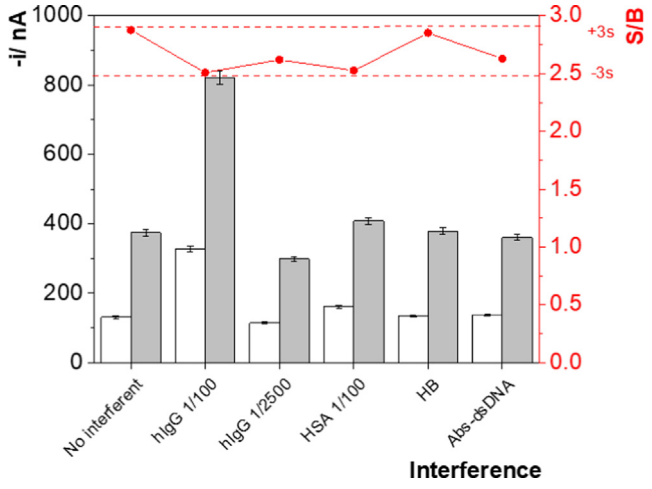


Fig. 3. Comparison of the amperometric responses provided by the developed bioplatforms for 0 and 2.5 ng mL^{-1} AQP4-Abs prepared in the absence and in the presence of 0.01 mg mL^{-1} and 0.0004 mg mL^{-1} hIgG, 0.5 mg mL^{-1} HSA, 5 mg mL^{-1} HB and 25 IU mL^{-1} dsDNA-Abs. Bars 1/100 and 1/2,500 correspond to the measurements for hIgG and HSA solutions with the concentrations existing in 100 and 2,500 times-diluted serum samples.

In addition, the bioplatforms were applied to the analysis of serum samples from healthy individuals and from patients diagnosed with SLE and AD. The potential existence of matrix effect in these samples was tested. The comparison of the slope value corresponding to the calibration plots constructed with standards (96 ± 4 nA^{-1}) with that constructed in 2,500-fold diluted sera (mean slope value = 91 ± 7 nA^{-1}) using the Student's t-test (t_{exp} = 0.310 < t_{tab} = 2.776) indicated that no apparent matrix effect occurred under the mentioned conditions [32]. It is important to note that according to the experimental results, there was a matrix effect working with lower dilutions of the samples. Therefore, the concentration of endogenous AQP4-Abs in the diluted serum samples could be calculated from the simple interpolation of the responses obtained for the samples into the calibration prepared with standards (Fig. 3). Fig. 4 shows the obtained results and these are compared in **Table S2** (in the Supporting Information) with those resulting from using the same immunoreagents with the ELISA method. The statistical comparison (t_{exp} was in all cases lower than t_{tab} = 2.776, α = 0.05; n = 4 [33]) and the correlation plot (r^2 = 0.999; slope = 1.000 ± 0.002; intercept = 4 ± 8) confirmed that similar results were obtained using both methodologies.

The obtained results demonstrated that the determination of AQP4-Abs in serum with the developed bioplatform allowed discrimination between healthy individuals and patients diagnosed with AD and SLE. Moreover, such discrimination was also possible between SLE (with a notably larger AQP4-Abs endogenous content, in agreement with an autoimmune disease) and AD patients. Although there are no data in the literature on the AQP4-Abs concentration in AD patients, the elevated level found may be justified by the altered distribution and expression of AQP4 reported in AD populations and animal models [34,35]. The obtained results support the suitability of the developed bioplatform to validate the

Table 2
AQP4-Abs concentration (in U mL^{-1}) determined with the developed bioplatform (n = 3) in control serum samples provided in the AQP4-Abs ELISA Version 2 Kit.

Control sample	Bioplatform	Specified in the commercial kit
Negative	0.15 ± 0.02; RSD = 5.6%	0.15
Positive I	12.0 ± 0.9; RSD = 4.3%	12.0
Positive II	28 ± 3; RSD = 5.6%	28

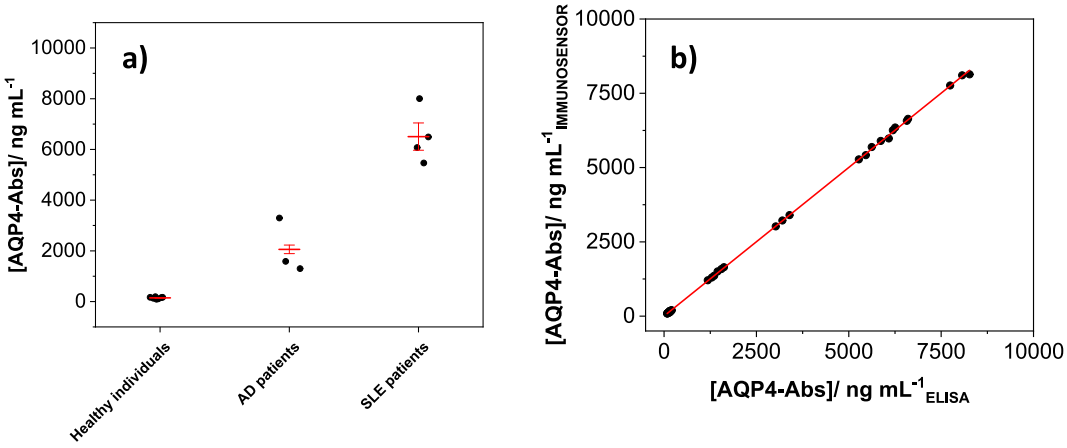


Fig. 4. AQP4-Abs concentrations obtained with the developed bioplatform for serum samples grouped into pools of healthy individuals and AD and SLE patients (a). Correlation plot between the AQP4-Abs concentrations obtained with the bioplatform and the ELISA method in the serum samples (b).

clinical potential of AQP4-Abs as a new target in relevant diseases, such as SLE and AD, where the AQP4-Abs concentration in serum is considerably smaller than that reported for NMO. In fact, the cut-off value established for NMO in serum is 3.0 U mL^{-1} or 260.9 μg mL^{-1} [36,37] with concentrations reported for patients as high as 13.984 ± 5.981 mg mL^{-1} [38]). It is important to note that not all commercially available ELISA kits possess the sufficient sensitivity to allow measurements for low AQP4-Abs concentrations (**Table S1** in the Supporting Information).

4. Conclusions

This work reports the first electrochemical bioplatform described to date for the determination of AQP4-Abs. The strategy is based on the selective capture of the Abs on MBs modified with the b-AQP4, the enzymatic labelling of the attached IgGs with an HRP-antihIgG and the amperometric transduction using the H_2O_2/HQ system after magnetic capture of the resulting bioconjugates on SPCEs. The developed bioplatform provides results with a high reproducibility and selectivity, and exhibits a better sensitivity than that claimed for the only biosensor reported to date for the determination of these specific antibodies, as well as that reported for commercially available ELISA kits. The suitability and applicability of the developed bioplatform has been demonstrated for the analysis of certified and real serum samples from healthy individuals and patients diagnosed with AD and SLE diseases, allowing clear discrimination between the 3 subject groups and providing results in agreement with those obtained by applying an ELISA method using the same immunoreagents. The excellent sensitivity achieved makes the bioplatform ideal for the diagnosis of diseases both showing high levels of these antibodies (such as NMO where AQP4-Abs are already clinically accepted diagnostic biomarkers), and others with less deregulation. The simplicity, affordable cost, reduced testing time and point-of-care applicability also make the developed bioplatform very promising for use by low-skilled personnel in decentralized or under-resourced settings.

CRediT authorship contribution statement

Beatriz Arévalo: Methodology, Investigation, Writing – review & editing, Writing – original draft. **Marina Blázquez:** Methodology, Investigation. **Verónica Serafín:** Methodology, Investigation, Writing – review & editing. **Ana Montero-Calle:** Investigation, Resources. **Miguel Calero:** Conceptualization, Supervision, Resources, Funding acquisition. **Alejandro Valverde:** Methodology, Investigation. **Rodrigo Barderas:** Conceptualization, Supervision, Resources, Funding acquisition. **Susana Campuzano:** Conceptualization, Supervision, Resources, Writing – review & editing, Funding acquisition. **Paloma Yáñez-Sedeño:** Conceptualization, Supervision, Resources, Writing – review & editing, Funding acquisition. **José M. Pingarrón:** Supervision, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioelechem.2021.108041.

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