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ABSTRACT

This work describes the use of layered double hydroxides (LDHs) for the immobilisation of acetylcholinesterase (AChE) on insulator/semiconductor solid supports. Different LDHs have been synthesised by a co-precipitation method. Afterwards, biohybrid materials based on AChE–LDH mixtures have been produced using wild and recombinant enzymes. Spectroscopic techniques have confirmed the LDH phase identity and the links created between the LDH and AChE. Spectrophotometric assays have demonstrated that most of the biohybrid materials are functional and stable. Several configurations have been used for AChE immobilisation. The highest catalytic responses have been observed when using wild enzyme and immobilising AChE–LDH mixtures on LDHs previously deposited on the solid supports. LDHs have been demonstrated to be suitable host matrices for AChE immobilisation on electrodes for the subsequent development of electrochemical biosensors.

1. Introduction

Biomolecule immobilisation is a crucial process in many biotechnological and bioengineering approaches. In particular, the immobilisation of enzymes is one of the key steps in the development of biosensors. The immobilisation method should not only guarantee that the enzyme is retained on the support, but also the active site of the enzyme should be accessible and maintain its functionality. Moreover, it should ensure a close proximity between the biological component and the transducer in order to obtain an efficient signal transfer. The immobilisation method may affect the performance of biosensors in terms of sensitivity, response time, stability and reproducibility. Consequently, there is a need to continuously explore new immobilisation strategies and optimise the existing protocols.

Acetylcholinesterase (AChE)-based biosensors have been widely developed and applied to the determination of pesticides in environmental monitoring and food quality control. Since their beginning, numerous methods have been used for AChE immobilisation, each one presenting advantages and drawbacks. The simplest immobilisation method was physical adsorption [1]. In this technique, based on weak interactions (e.g. hydrogen bonds, salt linkages and Van der Waals forces), the enzyme does not suffer from substantial modifications. However, enzyme desorption is usually a fact. Covalent binding using cross-linkers such as glutaraldehyde, carbodiimide/succinimide or aminopropyltriethoxysilanes have been used for AChE immobilisation on activated supports [2–7]. Although with this method the immobilisation stability increases, the functionality of the biomolecule may be compromised due to the strong bonds created. Another method for AChE immobilisation is the entrapment into polymeric or sol–gel matrices [8–12]. This method is interesting because of its simplicity and the flexible enzyme conformation into the network. Nevertheless, its limitations are the enzyme leakage and the existence of barriers that may limit the accessibility to the active site. The metal coordination chemistry has also been exploited for AChE immobilisation [13,14]. In this case, recombinant enzymes produced by genetic engineering incorporate hexa-His tails for their attachment to Ni-modified electrodes. The His residues are strategically located at appropriate positions of the enzyme sequence, at the opposite side from the active site in order to not alter the folding and functionality of the protein. This immobilisation method provides with oriented immobilisations, which may contribute to attain higher sensitivities and lower limits of detection. Nevertheless, the operational and storage stability may be reduced. A particular case of the exploitation of the metal coordination chemistry is the conjugation of recombinant AChE to Ni-modified magnetic particles [15]. The biosensor developed

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with this strategy provided with lower limits of detection than when using encapsulation into matrices. The affinity of sugars for Concanavalin A lectin has also been used as a method for AChE immobilisation [16,17]. The sugar moiety is naturally present in AChE and is generally located on areas that are not essential for its functionality.

LDHs, also known as hydrotalcite-like materials, are two-dimensional nanostructured anionic clays. These lamellar structures consist of main layers with two types of metallic cationic species and interlayer domains occupied by anionic species. Due to their large surface area, high anion exchange capacity, biocompatibility and tuneable surface and porosity properties, they have been used for multiple applications. One of them, since they can act as host structures for biomolecule immobilisation, is the development of biosensors [18–26].

In this work, the immobilisation of AChE through layered double hydroxides (LDHs) is proposed for the first time. Firstly, LDHs have been synthesised by a co-precipitation method using $NiCl_2$ or $CoCl_2$ and $AlCl_3$ or $FeCl_3$. Secondly, biohybrid materials based on LDHs and two different sources of AChE (wild and recombinant) have been then prepared. The structure of both the LDHs and the biohybrid materials has been then characterised by spectroscopy techniques. Finally, AChE immobilisation on solid supports has been investigated using several configurations and the enzyme functionality has been characterised by spectrophotometry.

2. Material and methods

2.1. Reagents and materials

Salts for the LDHs synthesis ($NiCl_2$, $FeCl_3$, $AlCl_3$, $CoCl_2$ and Na_2SO_4), acetylcholine iodide (AChI), acetylcholine chloride (AChCl), 5,5-dithiobis(2-nitrobenzoic acid) (DTNB) and components of buffers were purchased from Sigma (St. Quentin Fallavier, France). Two types of acetylcholinesterase (AChE), one wild from Sigma (**W**) and one recombinant produced by IMAGES laboratory (UPVD, Perpignan, France) (**R**), were used. The enzyme activity was expressed in U/mL, 1 U being defined as the amount of enzyme required to hydrolyse 1 μmol of substrate in 1 min at 25 °C. All solutions were prepared using milli-Q water obtained from a Millipore purification system (Bedford, MA, USA).

Cuvettes and microplates were obtained from Brand (Wertheim, Germany) and Nunc (Roskilde, Denmark), respectively. Wafers were obtained from the Centro Nacional de Microelectrónica (CNM, Barcelona, Spain). Insulator/semiconductor '100'-Oriented *p*-type Si wafers (diameter = 10 cm; cut into 1 cm × 1 cm squares) had a nominal resistivity of 4–40 Ω cm. The fabrication process started with a thermal oxidation process to grow a SiO_2 layer (78 nm) on *p*-type Si wafers at 950 °C. Then, a low-pressure chemical vapour deposition (LPCVD) Si_3N_4 layer (100 nm) was deposited on the SiO_2 at 800 °C. Prior to use, Si_3N_4 solid supports were cleaned by immersion into piranha solution (70% H_2SO_4:30% H_2O_2) for 15 min, rinsing with milli-Q water and drying under a nitrogen flow. Afterwards, they were immersed into a H_2SKCr_2 solution for 30 min, ultrasonicated in acetone for 3 min and in milli-Q water for 3 min, and finally dried under a nitrogen flow again. Screen-printed Co-phthalocyanine/carbon electrodes (DRP-410) with silver as a reference electrode and carbon as a counter electrode were purchased from Dropsens (Oviedo, Spain).

2.2. LDH and AChE–LDH synthesis

LDHs were prepared by a co-precipitation method [27]. Briefly, 100 mL of 0.1 M $NiCl_2$ or $CoCl_2$ and 50 mL of 0.1 M $AlCl_3$ or $FeCl_3$ solutions were mixed dropwise under stirring (1250 rpm) at 65 °C for 1 h. During mixing, pH was held constant at 11 in the case of NiAl and CoAl LDHs and at 11.5 in the case of NiFe and CoFe LDHs, by carefully adding a 2 M NaOH + 0.25 M Na_2SO_4 solution. Precipitates were matured for 16 h in the case of CoAl and 4 h for the rest of LDHs. LDHs were then filtrated, washed with ultrapure water, dried at 100 °C for 24 h and crushed. A final calcination step at 150 °C was included in the synthesis of the CoFe in order to obtain an appropriate LDH. LDHs aqueous solutions then prepared by dispersion in water at 2.5 mg/mL.

AChE–LDHs were prepared by mixing LDHs aqueous solutions and AChE solutions (at different concentrations in Phosphate Buffered Saline (PBS)). After a 3-h conditioning step at 4 °C, mixtures were ready to use.

2.3. AChE immobilisation on solid supports

A thin LDH (NiAl, NiFe, CoAl and CoFe) layer was deposited on clean Si_3N_4 solid supports by spin-coating (2500 rpm for 2 min). Then, 20 μL of AChE or AChE–LDH (10 mU) were spread on the LDH layer and kept at 4 °C overnight, providing with the corresponding AChE/LDH and AChE–LDH/LDH configurations, respectively. Prior to measurements, modified solid supports were immersed into PBS for 1 h and then carefully rinsed. Controls without LDH (using PBS instead) were always performed.

2.4. LDH, AChE/LDH and AChE–LDH structural characterisation

Power X-ray Diffraction (XRD) analysis of LDHs (NiAl, NiFe, CoAl and CoFe) was performed with an X'Pert Pro diffractometer (PANalytical, Almelo, The Netherlands) using Co Kα radiation (λ = 0.178901 nm) at 40 kV and 30 mA, and continued scanning mode. The patterns were recorded in a 2θ range from 7° to 80°, in 0.16° steps and 2 s per step.

Atomic Force Microscopy (AFM) Imaging of LDHs (NiAl, NiFe, CoAl and CoFe) was performed in tapping mode using a Dimension 3100 (Veeco) AFM equipment.

Attenuated Total Reflectance (ATR) using Fourier Transform Infra-Red (FTIR) spectra of AChE/LDHs (in solid mode) and AChE–LDHs (in liquid mode) were recorded using a QUINOX 55 (Bruker, Germany) spectrophotometer in the 4000–400 cm^{-1} range with 2 cm^{-1} resolution and averaging 128 scans.

2.5. AChE–LDH, AChE/LDH and AChE–LDH/LDH functional characterisation

To record the enzymatic activity of AChE–LDHs in solution, 20 μL of the mixtures (10 mU) were added to microtiter wells containing 580 μL PBS with 1% BSA (PBS–BSA), 100 μL of 10 mM AChI and 300 μL of 10 mM DTNB (both prepared with PBS–BSA), and incubated under stirring. Absorbance was recorded every 1.5 min during 15 min, using a Synergy HT microplate reader (BioTek Instruments, Inc., Winooski, VT, USA).

In the case of the modified solid supports (AChE/LDH and AChE–LDH/LDH), the protocol suffered from slight modifications: 600 μL PBS–BSA were used (since AChE or AChE–LDH were already immobilised on the support) and measurements were taken every 10 min for 1 h (modified solid supports were withdrew every measurement and immersed again to continue with the colour development until next measurement).

3. Results and discussion

3.1. LDH, AChE/LDH and AChE–LDH structural characterisation

The XRD patterns, shown in Fig. 1, confirmed the crystalline phase identity of the LDHs. The XRD patterns for CoAl and CoFe

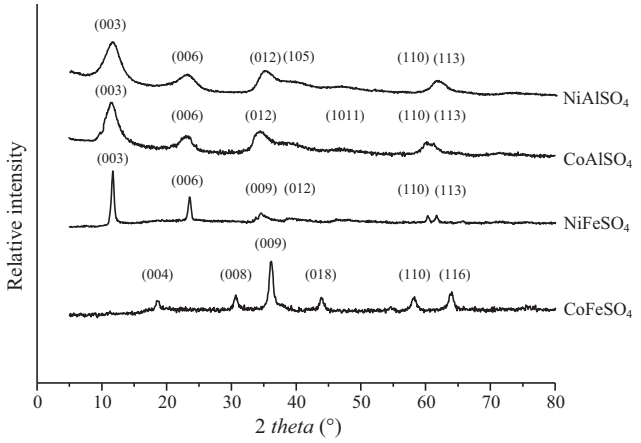


Fig. 1. XRD patterns for different LDHs prepared by co-precipitation.

LDHs showed symmetrical peaks, indicating that the obtained materials had crystallised with an ordered layered structure [28]. In the synthesis of the CoFe, a calcination step was necessary in order to obtain an appropriate LDH. The XRD pattern correlates with that reported by del Arco et al. [29], who described the spinel structure of CoFe formed upon calcination. The XRD patterns for NiFe LDHs indicated that reevesites had been synthesised [30]. The XRD patterns for *Rhomboédrique R3/m* NiAl LDHs showed a peak (0 0 6) at $2\theta = 25.92^{\circ}$, which is characteristic of takovites [31]. The composition of the LDHs was: $Ni_{2.90}Al(OH)_xSO_4$, $Ni_{2.22}Fe(OH)_xSO_4$, $Co_{1.57}Al(OH)_xSO_4$ et $Co_{2.23}Fe(OH)_xSO_4$, x being different for each LDH. Consequently, the molar ratio was 2 for NiFe, CoAl and CoFe and 3 for NiAl.

Concerning AFM experiments, the images showed a roughness of less than 1 nm, informing about the LDH particle size and confirming the uniformity of all LDHs.

FTIR analysis was performed using AChE from IMAGES (R). FTIR spectra of the enzyme immobilised on thin LDH layers (AChE/LDH) demonstrated the presence of covalent links at the interface between the enzyme and the LDH. No significant differences were observed among LDHs. Table 1 summarises the bending vibration FTIR peaks/ranges and the nature of the corresponding covalent links. Thiol links (S–H) at 2527 cm^{-1} suggest that the polar group of cysteines present into or at the periphery of the AChE could be linked to the LDHs. In the same way, as the bands at 1564–1512, 1480–1391 and 860–662 cm^{-1} and the peak at 608 cm^{-1} indicate, amine groups of AChE formed covalent links with the LDHs. The medium intensity band observed at 1235 cm^{-1} is one of the asymmetric stretching modes of amide III/CH2, certainly due to the vibration between glycine backbones [32,33]. The C=O links may be due to the protein secondary structure, which may have changed after the enzyme immobilisation [34]. Nevertheless, they may be also due to the interaction between carbon of amino acids and LDH hydroxides. In this case, the observed links could indicate that the AChE has been not only immobilised on the LDH surface, but also incorporated inside, at least at the top layer of the LDH. In fact, it is not possible to identify which of the two effects are present or even if it is a combination of both.

Table 1
Bending vibration peaks and ranges and the corresponding links obtained in the FTIR analysis of recombinant AChE immobilised on LDHs.

Bending vibration peaks and ranges (cm^{-1})	Corresponding links
3372	C–H
2527	S–H
2161	C=O
2025–1979	C–NO_2 (secondary)
1765–1710	OH
1658–1631	C=O
1564–1512, 1480–1391	N–H
1235	N–C (primary)
1000–890	=C–H
860–662	N–H (primary)
608	N–H (secondary)

Table 2
Bending vibration peaks and ranges and the corresponding links obtained in the FTIR analysis of recombinant AChE–LDH biohybrid materials in liquid mode.

Bending vibration peaks and ranges (cm^{-1})	Corresponding links
3372	C–H
2280–2265	C–NO_2
2025–1979	C–NO_2 (secondary)
1765–1710	OH
1610–1492, 1480–1320	N–H
720, 662	N–H (primary)
608	N–H (secondary)

In one of the immobilisation configurations, AChE–LDH mixtures were deposited on LDH-modified solid supports. This strategy was pursued in order to assure that the enzyme can freely interact with the LDH without restrictions due to the immobilisation on the support, which could limit its functionality. To characterise these AChE–LDHs prior to their immobilisation, FTIR spectra of the mixtures were recorded in liquid mode. No significant differences were observed among AChE–LDHs. Table 2 summarises the bending vibration FTIR peaks/ranges and the nature of the corresponding covalent links. Results differ from those observed when AChE was directly immobilised on LDHs. In this case, most of the covalent links were established between charge compensator anions of LDHs and amine or hydroxide groups from AChE. Unlike in the previous configuration, C=O and S–H links were not observed in the AChE–LDH mixtures. The absence of these links entails that the secondary structure is not, or at least is less, involved in the interaction with the LDH. The AChE–LDH mixture can be considered as a biohybrid material with a lower global energy than AChE/LDH, and thus more stable from a structural point of view and possibly more active from a functional point of view.

The structural characterisation of AChE/LDH and AChE–LDH and the multiple covalent links observed demonstrate the capacity of AChE and LDH to react with each other, forming a stable biohybrid material. In next sections, experiments pursued the characterisation of the functionality (catalytic activity) of the enzyme in AChE/LDH and AChE–LDH/LDH configurations.

3.2. AChE–LDH, AChE/LDH and AChE–LDH/LDH functional characterisation

The final goal of this work is to demonstrate that LDHs can be used for AChE immobilisation on electrodes for the future development of electrochemical biosensors. Consequently, it is necessary to demonstrate not only that the enzyme has been successfully immobilised, but also that it continues being functional after the immobilisation process, i.e. it has retained its catalytic activity. With this purpose, spectrophotometric measurements have been performed, which have contributed to compare the effect of the enzyme nature in the catalytic activity of the AChE–LDH mixtures and on the immobilisation yields, to evaluate the stability of the enzyme activity of the biohybrid materials in solution, and to choose the best AChE immobilisation configuration.

3.2.1. Comparison between AChE sources in AChE–LDH biohybrid materials

AChE from IMAGES (AChE(R)) contains a hexa-His tag at the C terminus, introduced to the enzyme by genetic engineering. His tails have been usually exploited in protein purification and immo-

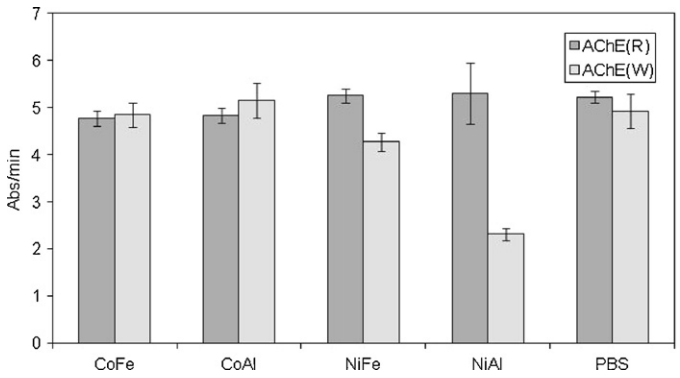


Fig. 2. ABS/min attained with AChE–LDH biohybrid materials in solution using recombinant (R) and wild (W) enzyme and different LDHs. PBS bar indicates control without LDH.

bilisation protocols, due to the affinity of metals towards these residues through the coordination chemistry. One of our hypothesis was that the His tail could be improving the links between AChE and LDHs. Consequently, AChE–LDH mixtures and the corresponding modified supports were prepared with recombinant AChE (AChE(R)) and wild AChE from Sigma (AChE(W)) (without the His tail), and the catalytic rates were compared.

Fig. 2 plots the enzyme activity rates achieved with different AChE–LDHs in solution. Results show that all the AChE–LDHs prepared with AChE(R) presented similar activity rates (and similar to control experiments with AChE(R) in PBS). In the case of AChE(W), values obtained with CoAl and CoFe were similar to those in PBS, but NiFe showed slightly lower activity rates and NiAl drastically lower rates. The trend observed with AChE(W)-modified supports was: **AChE(W)–CoAl** ∼ **AChE(W)–CoFe** ∼ **AChE(W)–PBS** > **AChE(W)-NiFe** ≫ **AChE(W)–NiAl**. Since this experiment was performed with AChE–LDHs in solution, it is sure that all microtiter wells contain the same enzyme amount. Consequently, the differences in the enzyme activity rates are certainly due to the effects of the LDHs on the enzyme activity. On the one hand, the bonds created may alter the protein folding and restrict the enzyme functionality or, on the contrary, may open the way towards the active site making it more accessible. On the other hand, metals may have an inherent activating/inhibiting power on the activity of some enzymes. Taking the case of the AChE(W)–NiAl mixture, it is clear that the low catalytic response cannot be due to only one of the metals of the LDH (low yields would have also been observed with AChE(W)–CoAl or AChE(W)–NiFe), but to a combination of both and the structure that is consequently formed. Moreover, the differences observed between AChE(R) and AChE(W) indicate that the enzyme nature has a decisive role in the formation, the functionality and the stability of the biohybrid material.

3.2.2. Stability of AChE–LDHs biohybrid materials

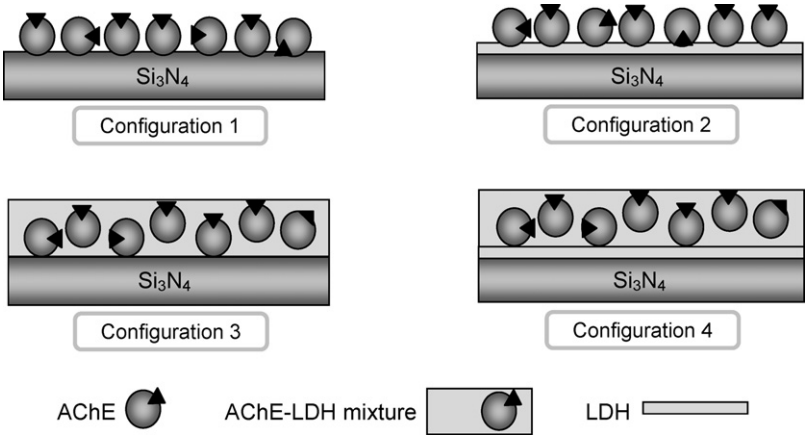
In order to know if, once synthesised, AChE–LDH biohybrid materials could be stored at 4 °C until use, their enzymatic activity in solution was recorded after several storage times (0, 15, 33, 57 and 141 h). In this experiment, wild AChE from Sigma (AChE(W)) was used. Results showed a very similar behaviour for AChE–CoFe, AChE–CoAl and AChE–NiFe. These biohybrid materials retained 90% of the initial enzyme activity after 15, 33 and 57 h, decreasing to 80% after 141 h. In the case of AChE–NiAl, the initial enzyme activity was lower than that attained with the other LDHs (as already observed in the previous section). This lower initial enzyme activity was probably responsible for the lower stability, being 50% after 15 h, 30% after 33 h, and negligible at the following storage times.

3.2.3. Choice of the best AChE-modified support configuration

In order to optimise the development of AChE-modified supports for the future biosensor development, different configurations were tested for the enzyme immobilisation (Scheme 1), using the same amount of enzyme in order to compare them. Configuration 1 was a control with the enzyme simply adsorbed on the solid support (AChE/SS); in configuration 2 the enzyme was immobilised on a previously deposited LDH (AChE/LDH/SS); in configuration 3 the enzyme–LDH mixture was immobilised on the solid support (AChE–LDH/SS); and in configuration 4 the enzyme–LDH mixture was immobilised on a previously deposited LDH (AChE–LDH/LDH/SS). In this experiment, wild AChE from Sigma (AChE(W)) and CoFe as LDH were used.

Fig. 3 shows the enzymatic activity attained using the different configurations. No significant differences were observed among the 3 first configurations, which provided with low enzyme activity rates. This result indicates that the LDHs present in configurations 2 and 3 are not providing with higher immobilisation yields than that attained with a simply enzyme adsorption (configuration 1). Nevertheless, it may be possible that the AChE is better immobilised when using LDHs than when simply adsorbed, but that the links created during the immobilisation process decrease its catalytic activity.

Configuration 4 provided with the highest enzyme activity rates. The difference between configuration 2 and 4 is only the immobilisation of AChE or AChE–LDH, respectively. Consequently, we hypothesise that the LDH present into the AChE–LDH mixture could be creating links with the previously deposited LDH, and thus improving the immobilisation yield. In other words, it seems to be more efficient to immobilise the enzyme already linked to



Scheme 1. Configurations used for AChE immobilisation.

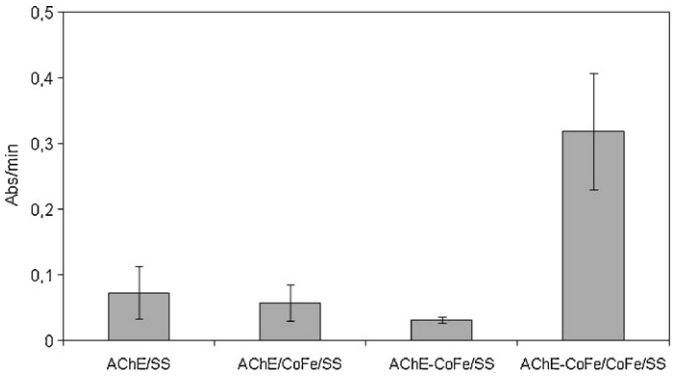


Fig. 3. ABS/min attained with different configurations used for the AChE immobilisation with wild enzyme and CoFe as LDH.

the LDH (i.e. enzyme encapsulated inside the LDH) than to immobilise free enzyme. It may be possible that in the encapsulation the enzyme feels freer to create comfortable links for its catalytic activity. Comparing configurations 3 and 4 (without and with a previously deposited LDH, respectively), the difference in the enzyme activity rate is certainly due to the fact that the LDH is better immobilised on the solid support when depositing it by spin coating (step only possible when depositing LDH without enzyme). This spin coating process confers an ordered LDH immobilisation, which contributes to stabilise the layer from a possible desorption. Following experiments were performed with configuration 4.

3.2.4. Comparison between AChE sources in AChE-modified supports

Biohybrid materials were immobilised on LDH-modified solid supports according to configuration 4. Fig. 4 shows the enzyme activity values attained with the AChE-modified supports using different LDHs. Compared with the biohybrid materials in solution, catalytic rates attained with modified supports were lower (enzymatic activity of 5% respect to the activity corresponding to the total spread enzyme). In fact, 45% enzyme activity was detected in the PBS solution where the modified supports had been immersed for conditioning after construction, proving that not all the enzyme molecules had been immobilised (added to a possible enzyme leaking after immobilisation). It is also necessary to take into account that, once the enzyme immobilised, the enzyme functionality may decrease due to conformational restrictions and steric impediments. This would explain that not 55% but 5% of the enzymatic activity was detected in the modified supports. The imbalance in enzyme activities is certainly due to the different enzyme environmental conditions (in solution and immobilised).

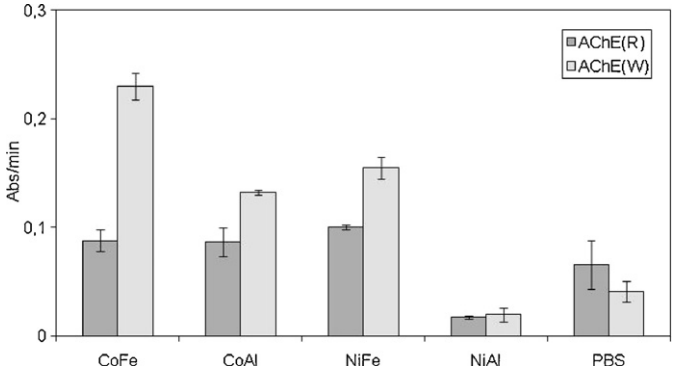


Fig. 4. ABS/min attained with configuration 4 for AChE immobilisation using recombinant (R) and wild (W) enzyme and different LDHs. PBS bar indicates control without LDH (i.e. configuration 1).

Solid supports modified with AChE(W)–LDH presented higher catalytic yields than the AChE(R)-LDH corresponding ones. Although we had hypothesised that the His tail could be contributing to better immobilise the enzyme, this metal coordination link (if present) could be producing a counterproductive effect, restricting the enzyme freedom and thus decreasing the catalytic rates. The AChE(W)–LDH supports (except the ones prepared with NiAl LDH) provided with immobilisation yields significantly different from that achieved with a simply enzyme adsorption, whereas the difference in the case of AChE(R)–LDH supports was not as evident.

In what concerns AChE(R), since no differences were observed among AChE–LDH biohybrid materials in solution, the differences between AChE-modified supports are certainly due to the immobilisation process. The trend observed was: **AChE(R)–CoFe ~ AChE(R)–CoAl ~ AChE(R)–NiFe > AChE(R)-PBS > AChE(R)–NiAl**. As demonstrated in the previous section for AChE(W), the immobilisation using AChE–LDH biohybrid materials (configuration 4) was better than when using free enzyme (configuration 1, PBS bar). No significant differences were observed between CoFe, CoAl and NiFe. However, the AChE(R)–NiAl modified supports showed significantly lower catalytic rates. It seems that the enzyme immobilisation yield is lower or that the NiAl layer previously deposited on the solid support is not orientating the enzyme properly, restricting its enzyme activity. Since appropriate activity rates were detected when using NiFe and CoAl, we cannot assign Ni or Al as responsible for the lower values attained with NiAl, but a combination of both.

When using AChE(W), the trend observed was: **AChE(W)–CoFe > AChE(W)–NiFe > AChE(W)–CoAl > AChE(W)–PBS > AChE(W)–NiAl**. Since the enzyme activities of the biohybrid materials were standardised before the construction of the modified supports (with the exception of AChE(R)–NiAl, whose low activity made not possible the standardisation), the differences observed are due to the immobilisation process.

4. Conclusions

This work reports the use of LDHs for AChE immobilisation for the first time. XRD patterns confirmed the crystallinity and phase identity of LDHs. FTIR analysis demonstrated that links were created between these LDHs and AChE, both when immobilising the enzyme on a LDH previously deposited on a solid support and when synthesising biohybrid materials based on the corresponding mixtures in solution. Nevertheless, differences in the nature of the covalent bonds were observed.

Regarding the functionality of AChE–LDH biohybrid materials, none of the tested LDHs affected the catalytic activity of the recombinant enzyme. However, the wild enzyme was slightly inhibited by NiFe and drastically inhibited by NiAl. It is evident that the LDH type, the enzyme source and nature, and the links created play important roles in the catalytic activity of the biohybrid materials, both in solution and immobilised.

Regarding AChE immobilisation of solid supports, the highest catalytic yields were obtained when depositing AChE–LDH biohybrid materials on LDH-modified supports. The LDH present into the mixture is probably creating links with the previously deposited LDH, which may favour the enzyme immobilisation and catalytic activity. Also higher were the catalytic rates provided by the wild enzyme. The links created with the hexa-His tail of the recombinant enzyme, if any, could be restricting its appropriate folding and subsequent functionality.

In conclusion, LDHs have been demonstrated to be an appropriate host matrix for AChE immobilisation on solid supports, suggesting their possible application to the development of electrochemical biosensors.

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