

A printed SWCNT electrode modified with polycatechol and lysozyme for capacitive detection of α -lactalbumin

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Abstract The authors describe an electrochemical sensor for the breast cancer marker α -lactalbumin (α LA). It is based on the use of printed single-walled carbon nanotube (SWCNT) electrodes that were modified with polycatechol. Impedance-derived electrochemical capacitance spectroscopy (ECS) is applied for detection at an applied potential of -0.14 V vs. Ag/AgCl reference electrode. The electrode was prepared in a two-step process. First, a dispersion of SWCNTs was drop-cast onto the surface of a poly(ethylene terephthalate) substrate to act as the working electrode. Next, catechol was electrochemically polymerized on the SWCNTs, prior to the immobilization of lysozyme. The strong interaction between lysozyme and α LA induced changes in the redox capacitance which are detected by ECS. The latter shows the device to be capable of detecting α LA in the 20 to 80 ng·mL⁻¹ concentration range. The limit of detection is 9.7 ng·mL⁻¹ at an S/N ratio of 3. The device was used to detect α LA in human blood serum with good recovery results.

Keywords Breast cancer · ECS · Single-walled carbon nanotubes · Polycatechol · Impedance · Lysozyme · Biosensor

Introduction

Nanomaterials have been shown to improve the performance of biosensors and to lower their detection limits due to their

unique physicochemical properties [1]. Electrochemical nanobiosensors are attracting increasing interests in several fields especially analytical and bioanalytical chemistry due to full compliance with ASSURED criteria [2–4].

Breast cancer is probably the most common form of cancer present within the female gender. The five-year survival rates are over 90% if the cancer is detected in its earliest stages [5]. Currently, intensive research efforts are devoted to find new methods of curing this form of cancer [6] and other non-invasive approaches for its early diagnosis [7]. Biosensors are expected to allow fast and reliable diagnostics that will result *in fine* in the popularization of the diagnostics and reduce the mortality rates. Detection of few circulating tumor cells in the blood stream and several specific proteins as cancer biomarkers are of paramount importance to allow the early-stage detection of the breast cancer [8]. Among these proteins, the most promising biomarkers are the vascular endothelial growth factor (VEGF) [9] and human epidermal growth factor receptor (HER2) [10]. Another interesting biomarker is α LA, which is a breast-specific differentiation protein present at high levels in the majority of human breast carcinomas [11]. The serum level of α LA is about 23.4 ± 5.6 ng·mL⁻¹ in sera of patients with breast cancer. It is worthy to note that the high levels of this protein in serum could be assigned also to pregnancy or some gynecological cancers [12].

The goal of the present work is to report the elaboration of a new nanobiosensing platform for the detection of α LA. The crucial steps in the elaboration of this biosensor were the choice of the probe and the analytical technique of detection. In our case, to perform the detection, we chose lysozyme (LYS) instead of using α LA monoclonal or polyclonal antibody due to the strong interaction existing between these two proteins (LYS and α LA) [13]. In fact, α LA has 40% of similarities in amino acid sequence with LYS and it has a closer spatial structure

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and gene organization [14]. Moreover, lysozyme is cost effective compared to α LA antibody and very easy to extract from hen egg white.

Enzyme immunoassay (ELISA) and fluorescence-linked immunosorbent assay (FLISA), which are based on the antigen-antibody reaction, are the most common techniques used to detect α LA [15]. Interestingly, it was reported that capacitance of surface-confined ferrocene molecules can be used to detect biomolecules [16]. In contrast to the traditional impedimetric method, no redox probe (or redox electrochemical pair) is added to the screened solution thus making the electrochemical capacitance spectroscopy experimentally adequate, and likely to be equally applicable to a broad range of target/receptor combinations in every potential and a suitable platform for multiplexing applications [17]. We aimed to apply similar treatment to our system.

Materials and methods

Reagents and apparatus

Catechol (>99%), lysozyme (from chicken egg white), α -lactalbumin (from human milk >95%), human serum, chitosan medium molecular weight (M_v 40,000–60,000), acetic acid and SWCNTs (with typical diameter of 0.7–0.9 nm and the purity is about 90%) were purchased from Sigma-Aldrich (www.sigmaaldrich.com). All reagents were of analytical grade and used without further purification. Phosphate-buffered saline (PBS, 0.1 M) was prepared by dissolving KCl, NaCl, K_2HPO_4 and KH_2PO_4 in deionized water.

All electrochemical measurements, cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS), were conducted using a Metrohm Autolab PGSTAT M204 electrochemical work station (www.metrohm-autolab.com/) equipped with a FRA impedance module at room temperature and in PBS (pH 6.5). Experiments data were collected using NOVA® software. Homemade screen-printed carbon counter and Ag/AgCl reference electrodes were used to perform the electrochemical experiments. The electrodes were printed on poly(ethylene terephthalate) (PET) sheets using a DEK-248 semi-automatic stencil printer (www.dek.com/). Deionized water was used to prepare all solutions.

Atomic force microscope (AFM) images were collected, in contact mode, using a SPM controller and a Park Systems XE-70 atomic force microscope (Park Systems Japan, Tokyo, Japan).

Preparation of the nanobiosensor

SWCNT electrodes were prepared according to the previously reported method by Rabi et al. [18, 19]. Briefly, they were

prepared by drop-casting of 5 μ L of SWCNTs (1.5 mg·mL⁻¹) dispersed in 0.4% (m:m) chitosan solution onto an insulating PET polymer to be used as the working electrode. The chitosan solution was prepared by dissolving 4 mg of chitosan in 1 mL of deionized water containing 10 μ L of glacial acetic acid with the aid of an ultrasonic bath for 1 h. After drying the electrode at RT for 2 h, the catechol (CC) was electrochemically polymerized by 200 continuous scanning of CV in the potential window of -0.4 to +0.4 V from 100 μ M catechol solution at a scan rate of 100 mV·s⁻¹.

The nanobiosensor was prepared by modifying the CC/SWCNT electrode with hen egg white lysozyme. In fact, the electrode was incubated in the solution of lysozyme (10 mg·mL⁻¹ in deionized water) for 16 h in the fridge (4 °C) then rinsed thoroughly with deionized water before use. The scheme of the electrochemical biosensor is shown in Fig. 1.

ECS detection of α -lactalbumin

To perform the assay, the biosensor was incubated in PBS containing the analyte (α LA) for at least 10 min under gentle stirring. After that, the electrode was rinsed with deionized water and the impedance measurements were recorded in PBS used as an electrolyte. EIS measurements were carried out in the frequency range from 10⁵ to 0.1 Hz at an applied potential of -0.14 V with an amplitude modulation of 10 mV. Measurements were taken after 10 min incubation to let lysozyme interact with the target. Impedance-derived capacitance, C^* , is a complex function that can be obtained using Equation 1:

$$C^* = 1/i\omega Z^* \quad (1)$$

where $Z^* = Z' + iZ''$, Z' and Z'' are respectively the real and imaginary components of the Z^* complex impedance function, and ω is the angular frequency.

The real and imaginary capacitance components can be obtained using the following equations:

$$C' = \varphi Z^{\wedge} \quad (2)$$

$$C^{\wedge} = \varphi Z' \quad (3)$$

Where $\varphi = 1/\omega|Z|^2$ and $|Z|$ is the modulus of Z^* .

Results and discussion

Preparation of LYS/CC/SWCNT electrode

SWCNTs are widely employed nanomaterials in electrochemical biosensing [20–22]. They have a high conductivity, a large capacitance (180 F·g⁻¹ [23]), hydrophobic surface and

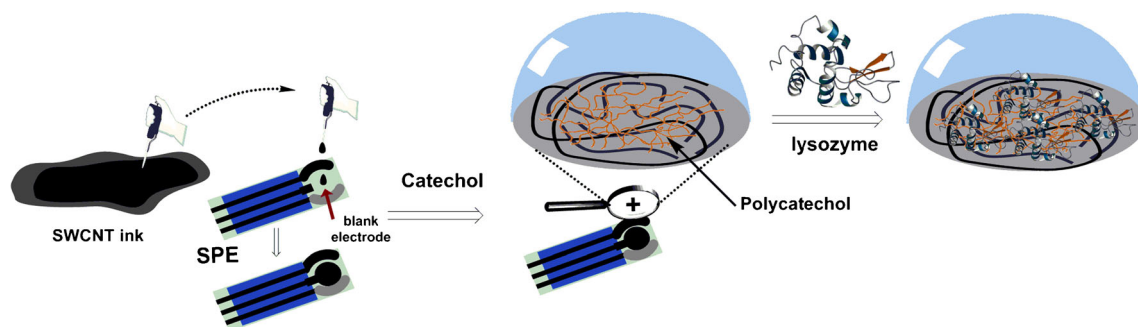


Fig. 1 Schematic representation of the nanobiosensor preparation

a large specific area, making of them a good support for the adsorption of enzymes [24, 25]. This combination generates a great nanomaterial for capacitive detection [26]. The lack of solubility of SWCNTs in aqueous media can be overcome by adding a little amount of chitosan, a cost-effective natural polymer, to the suspension as a stabilizer and for lysozyme immobilization [27]. Coating SWCNTs with redox-active polymer is a promising pathway to extend their applications in electronic devices [28, 29]. In our case, polycatechol acts as an electrochemical transducer since it undergoes electron-transfer at low potentials.

Figure 2 displays the different characterizations of the modified SWCNT electrodes. In the presence of catechol and after repetitive voltammetric cycling, two separate redox peaks appeared at different potentials denoting two different behaviors of the catechol (Fig. 2a). These

peaks can be attributed respectively to free (peak 1) and polymerized (peak 2) catechol since the former one was not present in the beginning of the cycling process. Moreover, after a gentle washing of the electrode with a PBS, we noticed the persistence of the response of the second peak and the loss of the one related to catechol (curve c, Fig. 2b). In solution, the free catechol is oxidized and reduced according to the redox reaction equation displayed in Scheme 1a.

Figure 2c shows the logarithm of the current versus the logarithm of scan rates for the catechol when it is oxidized in solution. The slopes of the anodic and cathodic peak currents are 0.6 ± 0.018 and 0.71 ± 0.017 , respectively. These values are more or less not far from 0.5, so we can conclude that the electron-transfer process is presumably diffusion-controlled.

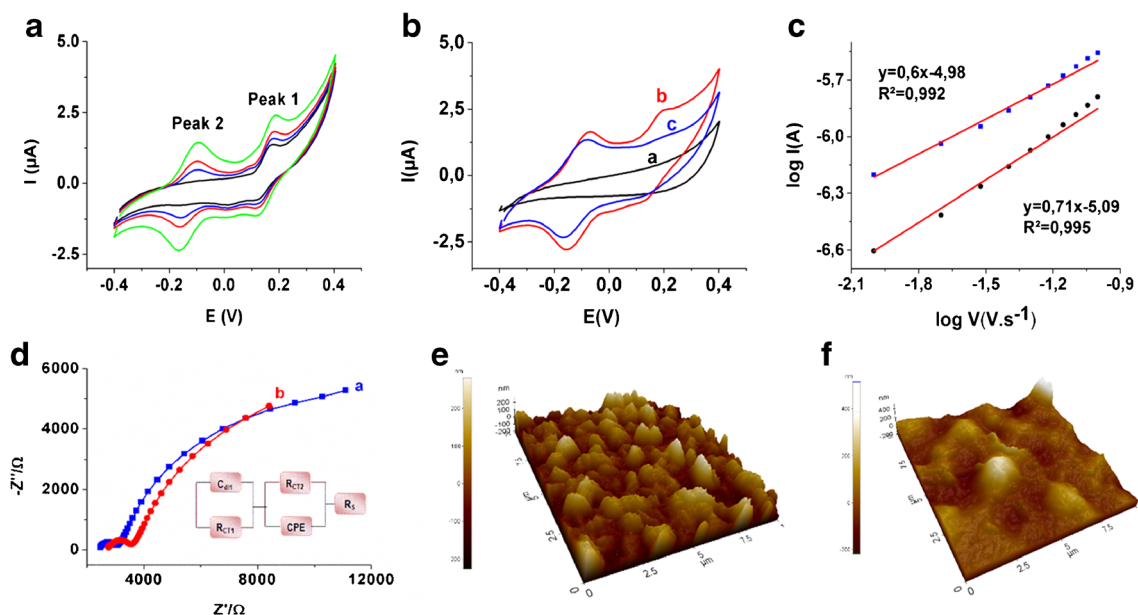
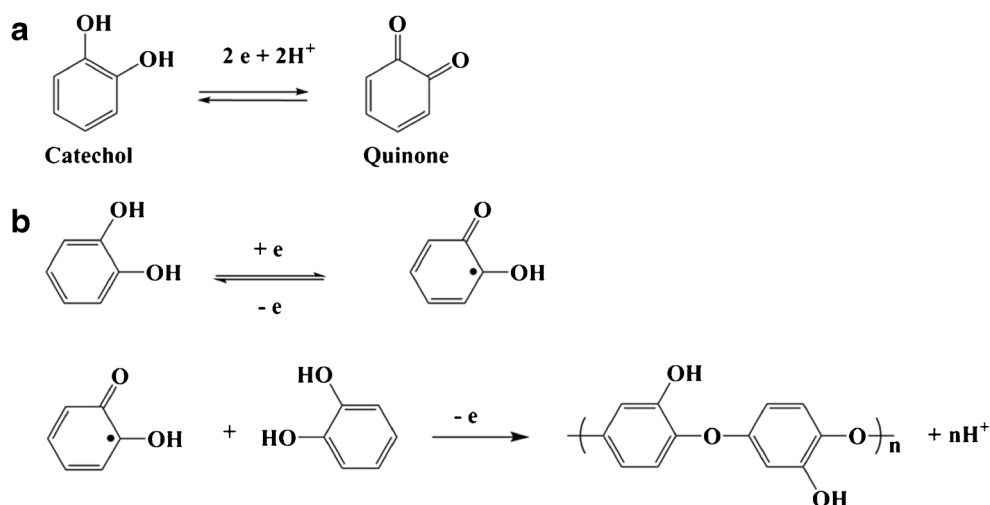


Fig. 2 **a** CVs of catechol at SWCNT electrode after repetitive scanning cycles (1(black), 10(blue), 20(red) and 50(green)) in 100 μM of catechol solution at 100 $\text{mV}\cdot\text{s}^{-1}$. **b** CVs of SWCNT electrode: (a) in PBS, (b) in 100 μM of catechol solution and (c) in PBS after electrodeposition of CC at 100 $\text{mV}\cdot\text{s}^{-1}$. **c** Plots of anodic and cathodic peak currents of CC/

SWCNT vs. logarithm of scan rate of peak 1. **d** Nyquist plots of (a) CC/SWCNT and (b) LYS/CC/SWCNT electrodes recorded in 0.1 M KCl solution containing 5 mM $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ at 0.15 V. **e** AFM image of CC/SWCNT and **f** LYS/CC/SWCNT electrodes

Scheme 1 a Redox behavior of catechol in solution and (b) mechanism of electrochemical polymerization of catechol



The electropolymerization of phenolic compounds is well-documented in literature [30–32]. The peak 2 appears during the second and subsequent voltammetric scans at anodic and cathodic potentials of -0.06 and -0.16 V, respectively. Its intensity increases with number of cycle suggesting the formation of a redox-active polymer on the surface of SWCNTs according to the mechanism displayed in Scheme 1b.

AFM was also used to characterize the modification of CC/SWCNT electrode with lysozyme (Fig. 2e–f). The difference in the electrode topologies before and after LYS deposition indicates the success of the surface modification. In fact, the thickness doubled (from 200 to 400 nm) after LYS addition and the surface become smoother. It is well-known that lysozyme have a very strong affinity to chitosan. Deacetylated chitosan (75 to 85%) interacts strongly with lysozyme. It was also reported that a fully deacetylated chitosan do not interact with lysozyme as observed by ^1H -NMR spectroscopy. However, a chitosan, with fraction of N-acetylated units = 0.04, induced significant ^1H shifts of several lysozyme resonances, demonstrating a specific interaction between lysozyme and de-N-acetylated units in the chitosan [33, 34].

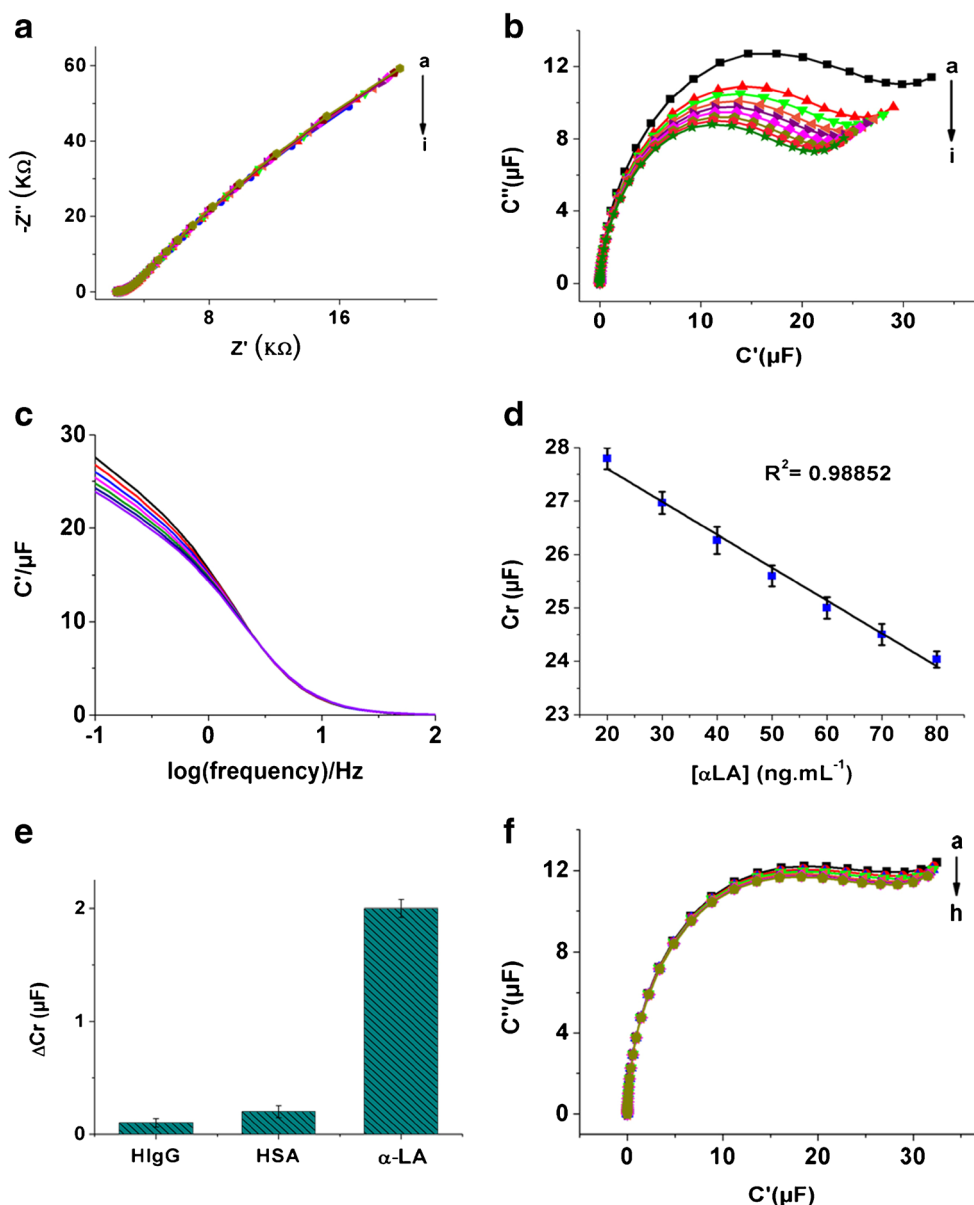
Furthermore, the electron-transfer kinetics of $[\text{Fe}(\text{CN})_6]^{3/4-}$ redox probe at CC/SWCNT and LYS/CC/SWCNT electrodes were compared using electrochemical impedance spectroscopy (Fig. 2d). EIS plots show two semi-circles, a complete and a non-complete one, that may correspond to the charge-transfer resistance levels at the CC/SWCNT interface and the electrolyte/LYS/CC or electrolyte/CC interface, respectively. The charge-transfer resistance (R_{CT}) of the CC/SWCNT electrode, which was equal to $546 \pm 36 \Omega$ ($n = 3$), increases to $613 \pm 25 \Omega$ ($n = 3$) ($\approx 12\%$ increase) to indicate that LYS was firmly adsorbed onto the CC/SWCNT electrode. The experimental data were fitted by using an equivalent circuit model (inset of Fig. 2d).

Detection of αLA

EIS measurements were used to follow the charge-transfer resistance after successive addition of αLA (Fig. 3a) but failed to show any significant variation. However, using the EIS-derived capacitance (*cf.* Eqs. 1–3), plotting the real component versus imaginary one showed a neat variation of the signal, which proves that the capacitance is a concentration-dependent on the amounts of αLA and is more sensitive than the impedance (Fig. 3b). Actually, capacitance measures the change in the redox capacitance (C_r) of the adsorbed redox film onto the electrode surface. Thus, the measured signal does not depend on charge-transfer resistance but on faradaic capacitive charging [35]. A control experiment performed without using catechol (changes in non-faradaic capacitance) does not show any appreciable variation of the capacitance. Moreover, it is important to note that C_r is maximal at the electrochemical half-wave potential of the electroactive specie [36]. In our case, this value is equal to -0.14 V and was used to carry out all the measurements.

Upon successive addition of αLA , the redox capacitance of LYS/CC/SWCNT electrode decreased (Fig. 3b). This is due to the electrostatic interactions between αLA and LYS and their ability to self-assemble into micrometric spheres. In fact, αLA is an acidic protein with a pI value near 4–5 and containing 123 amino acids while LYS is a basic protein with a pI value of 10.7 consisting of 130 aminoacids, which indicates that they can undergo with electrostatic interactions in well-defined conditions [37, 38]. Even knowing that C_r is obtained from the diameter of the semi-circle from Nyquist capacitive plot, this decrease of capacitance with the target concentration increasing was elucidated by plotting C_r as a function of αLA concentration as depicted in Fig. 3d. We further plotted the real capacitance component versus the logarithm of

Fig. 3 **a** Impedance Nyquist plots, **(b)** ECS Nyquist plots and **(c)** Real capacitance vs. logarithm of frequency of LYS/CC/SWCNT electrode at different concentrations of α LA (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50, (g) 60, (h) 70, (i) 80 $\text{ng}\cdot\text{mL}^{-1}$ α LA recorded in PBS at -0.14 V. **d** Calibration curve for α LA. **e** Selectivity experiments on LYS/CC/SWCNT electrode at -0.14 V for 50 $\text{ng}\cdot\text{mL}^{-1}$ of each analyte: human immunoglobulin G, human serum albumin and α -lactalbumin. **f** ECS Nyquist plots of LYS/SWCNT electrode at different concentrations of α LA (a) 0, (b) 10, (c) 20, (d) 30, (e) 40, (f) 50, (g) 60, (h) 70 $\text{ng}\cdot\text{mL}^{-1}$ α LA recorded in PBS at -0.14 V



the frequency to extract the Cr values (Fig. 3c) [39]. The bioelectrode can detect α -lactalbumin in the linear

range of 20–80 $\text{ng}\cdot\text{mL}^{-1}$ according to the following equation:

$$\text{Cr } (\mu\text{F}) = -0.06 \times [\alpha\text{LA}], \text{ng} \cdot \text{mL}^{-1} + 28.96 \quad (4) \text{ with a limit of detection of } 9.67 \text{ ng} \cdot \text{mL}^{-1} \\ (\text{S/N} = 3) \text{ and a limit of quantification } (\text{S/N} = 10) \text{ equal to } 29.03 \text{ ng} \cdot \text{mL}^{-1}.$$

In a further consolidation of the method to detect α LA, a control experiment was carried out without the use of catechol. Fig. 3f shows the ECS Nyquist plots of LYS/SWCNT electrode after addition of different concentrations of α LA in the absence of any redox species. The capacitance was quasi-invariable after each addition, which demonstrates that the redox capacitance is the one responsible for the detection.

This can be explained by the fact that the formation of bioaffinity complexes retards the interfacial electron-transfer kinetics. Thus, the presence of a redox center is essential in such technique as a detection tool [40].

We also compared our results to some other methods from literature used to detect α -lactalbumin (Table 1). The main strong points of our device that it uses a direct method for

Table 1 Comparison of the present work with other published works for α LA detection

Materials used	Method applied	LOD/sensitivity	references
α LA mAb	RIA	—/ 72%	[10]
α LA mAb/CdSe/ZnS QDs	FLISA	0.1 ng·mL ⁻¹ /—	[15]
α LA mAb	ELISA	—/ 0.128 ng·mL ⁻¹	[41]
H ₂ O ₂ /HQ/HRP/ α LA mAb/MBs/SPCE	amperometry	11 pg·mL ⁻¹ / --	[42]
Lysozyme/CC/SWCNT	ECS	9.7 ng·mL ⁻¹ /—	This work

RIA Radioimmunoassay, mAb Monoclonal antibody, HQ Hydroquinone, HRP Horsradish peroxidase, DQs quantum dots, MBs Magnetic beads, SPCE Screen-printed carbon electrode

the detection and not a sandwich one. Besides, it avoids using expensive monoclonal antibodies and toxic nanoparticles such as cadmium-based QDs. Although, in our case, the limit of detection is higher, it remains sufficient to conveniently detect the analyte in human blood serum (average of 25 ng·mL⁻¹) and in cow milk (1.5 mg·mL⁻¹) [43].

Immunoglobulin G from human serum (hIgG) and human serum albumin (HSA), found in blood and extracellular fluid, were used to challenge the device in order to test its selectivity to the analyte. The results, shown in Fig. 3e, reveal that lysozyme interacts very well with α LA and is almost insensitive to the other species. Thus, the device can be used to analyze real samples. Furthermore, to evaluate the applicability of the prepared bioelectrode in real samples, we used it to detect α LA in human blood serum purchased from Sigma-Aldrich without performing any pretreatment. To obtain suitable to incubate the bioelectrode, the blood serum was diluted twice before use in order to obtain less viscous solutions. In preliminary experiments, α LA was not detectable in the diluted samples and different concentrations of α LA were therefore added. The determined values are close to those added in the second step. The recovery values are gathered in Table 2, they demonstrate that the method can be reasonably applied for physiological liquids.

Conclusion

We developed a new biosensor for α -lactalbumin detection in human blood without the need of α -lactalbumin antibody.

Table 2 Determination of α LA in human blood serum using LYZ/CC/SWCNT electrode

Samples	Serum (R)	Added (P) (ng·mL ^{-a})	Found (F)(ng·mL ^{-a})	RSD ^b (%)	Recovery ^c (%)
1	ND ^a	20.0	21.1	2.15	94.7
2	ND	30.0	30.9	3.00	97.0

^a ND not detected, ^b Relative standard deviation (RSD) of 3 measurements, ^c Recovery (%) = 100×[R + P]/[F]

Using impedance-derived electrochemical capacitance spectroscopy as a tool, we demonstrated that SWCNT electrode modified with catechol and lysozyme is an attractive platform to detect α -lactalbumin as a biomarker of breast cancer. The designed biosensor is cost effective and selective but still have some limitations related to the homogeneity of the electrode surface after drying. The level of α -LA in serum is predictive because it is indicative for breast cancer or some gynecological carcinomas. The bioelectrode may also be used to detect the analyte in cow milk since it is a common allergen.

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Compliance with ethical standards The author(s) declare that they have no competing interests.

References

- Sagadevan S, Periasamy M (2014) Recent trends in nanobiosensors and their applications-a review. *Rev Adv Mater Sci* 36:62
- Yang N, Chen X, Ren T, Zhang P, Yang D (2015) Carbon nanotube based biosensors. *Sensors Actuators B* 207:690
- Kumar S, Ahlawat W, Kumar R, Dilbaghi N (2015) Graphene, carbon nanotubes, zinc oxide and gold as elite nanomaterials for fabrication of nanobiosensors for healthcare. *Biosens Bioelectron* 70:498
- Jia L, Lawrence G, Balasubramanian VV, Choi G, Choy JH, Abdullah AM, Elzatahry A, Ariga K, Vinu A (2015) Highly ordered nanoporous carbon films with tunable pore diameters and their excellent sensing properties. *Chem Eur J* 21:697
- DeSantis C, Ma J, Bryan L, Jemal A (2014) Breast cancer statistics, 2013. *CA Cancer J Clin* 64:52
- Hayes DF (2016) Is breast cancer a curable disease? *J Oncol Practice* 12:13
- Azimzadeh M, Rahaiea M, Nasirizadeh N, Ashtari K, Manesh HN (2016) An electrochemical nanobiosensor for plasma miRNA-155, based on graphene oxide and gold nanorod, for early detection of breast cancer. *Biosens Bioelectron* 77:99
- Weigel MT, Dowsett M (2010) Current and emerging biomarkers in breast cancer: prognosis and prediction. *Enodcr Relat Cancer* 17: 245

9. Zhang SJ, Hu Y, Qian HL, Jiao SC, Liu ZF, Tao HT, Han L (2013) Expression and significance of ER, PR, VEGF, CA15-3, CA125 and CEA in judging the prognosis of breast cancer. *Asian Pac J Cancer Prev* 14:3937
10. Tabasi A, Noorbakhsh A, Sharifi S (2017) Reduced graphene oxide-chitosan-aptamer interface as new platform for ultrasensitive detection of human epidermal growth factor receptor 2. *Biosens Bioelectron* 95:117
11. Botelho ACF, Ferreira MC, França JL, França EL, França ACH (2012) Breastfeeding and its relationship with reduction of breast cancer: a review. *Asian Pac J Cancer Prev* 13:5327
12. Thean ET, Toh BH (1990) Serum human alpha-lactalbumin as a marker for breast cancer. *Br J Cancer* 61:773
13. Nigen M, Croguennec T, Renard D, Bouhallab S (2007) Temperature affects the supramolecular structures resulting from α -lactalbumin-lysozyme interaction. *Biochemistry* 46:1248
14. Qasba PK, Kumar S (1997) Molecular divergence of lysozymes and α -lactalbumin. *Crit Rev Biochem Mol Biol* 32:255
15. Yang A, Zheng Y, Long C, Chen H, Liu B, Li X, Yuan J, Cheng F (2014) Fluorescent immunosorbent assay for the detection of alpha lactalbumin in dairy products with monoclonal antibody bioconjugated with CdSe/ZnS quantum dots. *Food Chem* 150:73
16. Santos A, Carvalho FC, Barreira MCR, Bueno PR (2014) Impedance-derived electrochemical capacitance spectroscopy for the evaluation of lectin-glycoprotein binding affinity. *Biosens Bioelectron* 62:102
17. Fernandes FCB, Santos A, Martins DC, Góes MS, Bueno PR (2014) Comparing label free electrochemical impedimetric and capacitive biosensing architectures. *Biosens Bioelectron* 57:96
18. Rabti A, Martinez CCM, Pires LB, Raouafi N, Merkoçi A (2016) Ferrocene-functionalized graphene electrode for biosensing applications. *Anal Chim Acta* 926:28
19. Rabti A, Argoubi W, Raouafi N (2016) Enzymatic sensing of glucose in artificial saliva using a flat electrode consisting of a nanocomposite prepared from reduced graphene oxide, chitosan, nafion and glucose oxidase. *Microchim Acta* 183:1227
20. Tominaga M, Sasaki A, Togami M (2016) Bioelectrocatalytic oxygen reaction and chloride inhibition resistance of laccase immobilized on single-walled carbon nanotube and carbon paper electrodes. *Electrochimica Acta* 84:315
21. Eguílaz M, Gutierrez F, Domínguez JMG, Martínez MT, Rivas G (2016) Single-walled carbon nanotubes covalently functionalized with polytyrosine: a new material for the development of NADH-based biosensors. *Biosens Bioelectron* 86:308
22. Stojanović ZS, Mehmehi E, Kalcher K, Guzsvány V, Stanković DM (2016) SWCNT-modified carbon paste electrode as an electrochemical sensor for histamine determination in alcoholic beverages. *Food Anal Methods* 9:2701
23. Lew W, Dai L (2010) Carbon nanotube supercapacitors. In: Marulanda JM (ed) *Carbon nanotubes*, 1st edn. InTech, Rijeka, pp 563–593
24. Adhikari BR, Schraft H, Chen A (2017) High-performance enzyme entrapment platform facilitated by a cationic polymer for the efficient electrochemical sensing of ethanol. *Analyst* 142:2595
25. Pagán M, Suazo D, Toro N, Griebenow K (2015) A comparative study of different protein immobilization methods for the construction of an efficient nano-structured lactate oxidase-SWCNT-biosensor. *Biosens Bioelectron* 64:138
26. Snow ES, Perkins FK (2005) Capacitance and conductance of single-walled carbon nanotubes in the presence of chemical vapors. *Nano Lett* 15:2414
27. Xiao H, Zhu B, Wang D, Pang Y, He L, Ma X, Wang R, Jin C, Chen Y, Zhu X (2012) Photodynamic effects of chlorin e6 attached to single wall carbon nanotubes through noncovalent interactions. *Carbon* 50:1681
28. Mosch HLKS, Akintola O, Plass W, Hoepfner S, Schubert US, Ignaszak A (2016) The specific surface versus electrochemically active area of the carbon/polypyrrole capacitor: the correlation of ion dynamics studied by an electrochemical quartz crystal microbalance with BET surface. *Langmuir* 32:4440
29. Xiong S, Wei J, Jia P, Yang L, Ma J, Lu X (2011) Water-Processable polyaniline with covalently bonded single-walled carbon nanotubes: enhanced electrochromic properties and impedance analysis. *Appl Mater Interfaces* 3:782
30. Bai J, Bo X, Qi B, Guo L (2010) A novel Polycatechol/ordered mesoporous carbon composite film modified electrode and its Electrocatalytic application. *Electroanalysis* 22(15):1750
31. Sayyah SM, Khaliel AB, Azooz RE, Mohamed F (2011) Electropolymerization of some Ortho- substituted phenol derivatives on Pt-electrode from aqueous acidic solution; kinetics, Mechanism, electrochemical studies and characterization of the polymer obtained. In: Balcerzak ES (ed) *Electropolymerization*. InTech, Rijeka, pp 1–53. isbn:978-953-307-693-5
32. Marczewska B, Przegalinski M (2013) Poly(catechol) electroactive film and its electrochemical properties. *Synth Met* 182:33
33. Kristiansen A, Vårum KM, Grasdalen H (1998) The interactions between highly de-N-acetylated chitosans and lysozyme from chicken egg white studied by ¹H-NMR spectroscopy. *Eur J Biochem* 251:335
34. Fukamizo T, Yamaguchi T, Araki T, Torikata T, Kristiansen A, Vårum KM (2001) Binding of a highly de-N-acetylated chitosan to Japanese Pheasant lysozyme as measured by ¹H-NMR spectroscopy. *Biosci Biotechnol Biochem* 65:1766
35. Fernandes FCB, Góes MS, Davis JJ, Bueno PR (2013) Label free redox capacitive biosensing. *Biosens Bioelectron* 50:437
36. Lehr J, Fernandes FCB, Bueno PR, Davis JJ (2014) Label-free capacitive diagnostics: exploiting local redox probe state occupancy. *Anal Chem* 86:2559
37. Nigen M, Croguennec T, Bouhallab S (2009) Formation and stability of α -lactalbumin-lysozyme spherical particles: involvement of electrostatic forces. *Food Hydrocoll* 23:510
38. Nigen M, Gaillard C, Croguennec T, Madec M-N, Bouhallab S (2010) Dynamic and supramolecular organization of α -lactalbumin/lysozyme microspheres: microscopic study. *Biophys Chem* 146:30
39. Bueno PR, Davis JJ (2012) Elucidating redox-level dispersion and local dielectric effects within electroactive molecular films. *Anal Chem* 86:1997
40. Bueno PR, Mizzon G, Davis JJ (2012) Capacitance spectroscopy: a versatile approach to resolving the redox density of states and kinetics in redox-active self-assembled monolayers. *J Phys Chem B* 116:8822
41. Tao C, Zhang Q, Feng N, Shi D, Liu B (2016) Development of a colloidal gold immunochromatographic strip assay for simple and fast detection of human α -lactalbumin in genetically modified cow milk. *J Dairy Sci* 99:1773
42. Montiel VRV, Campuzano S, Rodríguez RMT, Reviejo AJ, Pingarrón JM (2016) Electrochemical magnetic beads-based immunosensing for the determination of α -lactalbumin in milk. *Food Chem* 213:595
43. Conrado LS, Veredas V, Nóbrega ES, Santana CC (2005) Concentration of α -lactalbumin from cow milk whey through expanded bed adsorption using a hydrophobic resin. *Braz J Chem Eng* 22:501