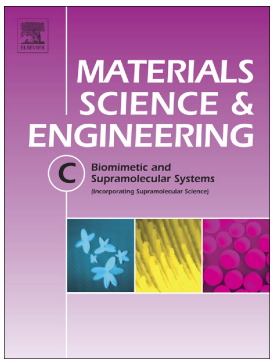

Voltammetric glucose biosensor based on glucose oxidase encapsulation in a chitosan-kappa-carrageenan polyelectrolyte complex

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PII: S0928-4931(18)31311-0
DOI: doi:10.1016/j.msec.2018.10.078
Reference: MSC 9004

To appear in: *Materials Science & Engineering C*

Received date: 8 May 2018
Revised date: 22 September 2018
Accepted date: 22 October 2018

Please cite this article as: Ilhem Rassas, Mohamed Braiek, Anne Bonhomme, Francois Bessuelle, Guy Rafin, Hatem Majdoub, Nicole Jaffrezic-Renault , Voltammetric glucose biosensor based on glucose oxidase encapsulation in a chitosan-kappa-carrageenan polyelectrolyte complex. Msc (2018), doi:10.1016/j.msec.2018.10.078

Voltammetric glucose biosensor based on glucose oxidase encapsulation in a chitosan-kappa-carrageenan polyelectrolyte complex.

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Abstract

In this work, a new design of voltammetric glucose biosensor, based on the encapsulation of glucose oxidase (GOx) in a chitosan/κ-carrageenan (CHIT/CAR) polyelectrolyte complex (PEC) using a simple coacervation process is presented. A conductometric monitoring of this is performed. Spectroscopic and morphological characterization of the PEC film encapsulating GOx is carried out. Compared to biosensors based on a chitosan film, a more sensitive voltammetric detection of glucose is obtained.

Using square wave voltammetry (SWV), the CHIT/CAR PEC based biosensor exhibits a wide linearity range from 5 µM to 7 mM glucose with a detection limit of 5 µM. Excellent selectivity against ascorbic acid, uric acid and urea is observed and the applicability of the biosensor for glucose detection in spiked saliva samples was demonstrated.

Keywords: chitosan; κ-carrageenan; polyelectrolyte complex; encapsulation, glucose oxidase, biosensor

1. Introduction

Over the past two decades, numerous studies have been reported on seaweed-derived polysaccharides for biomedical and biological applications (tissue engineering, drug delivery, wound healing, and biosensors). Alginate, carrageenan, fucoidan, and ulvan are widely used marine derived polysaccharides for biological and biomedical applications due to their biocompatibility and availability. Sulfated polysaccharides such as carrageenan show

promising applications in tissue engineering due to their capacity to induce important osteogenic, adipogenic, and chondrogenic differentiation in stem cells [1].

Polysaccharides are complex carbohydrate polymers consisting of more than 2 monosaccharides covalently linked by glycosidic linkages. Elementary motives of unbranched homo-polysaccharides such as chitosan and κ-carrageenan are presented in Figure 1. Chitosan belongs to the family of cationic polysaccharides and κ-carrageenan to the family of anionic polysaccharides.

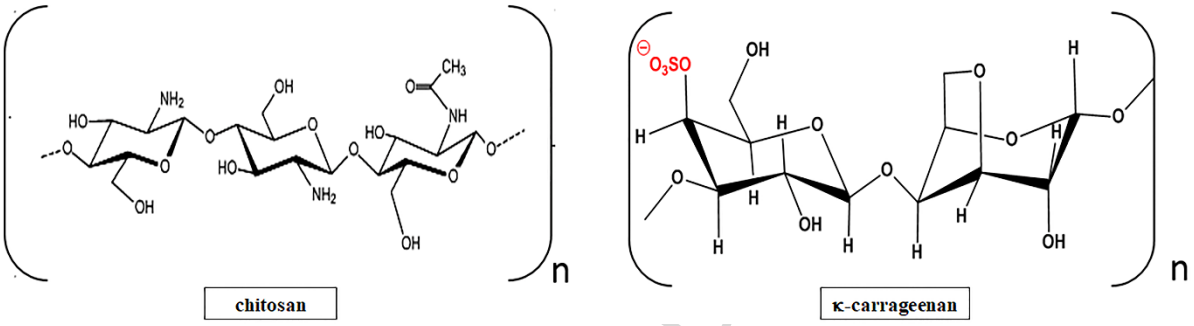


Figure 1 : Structures of elementary motifs of chitosan and κ-carrageenan

The complexation of oppositely charged polysaccharides (positively charged chitosan, due to charged amine groups and negatively charged κ-carrageenan, due to sulfate groups) is schematized (cf Fig. 2) as an association of flexible macromolecular chains into scrambled egg-like globules. The electrostatic interactions between oppositely charged groups cause a decrease of the total charge of the polyelectrolyte complex (PEC) in comparison with the initial carbohydrate macromolecules that leads to their shrinkage into tight globules [2]. This results in a decrease of solubility owing to the increased hydrophobicity that can lead to PEC precipitation, which constitutes a coacervation process.

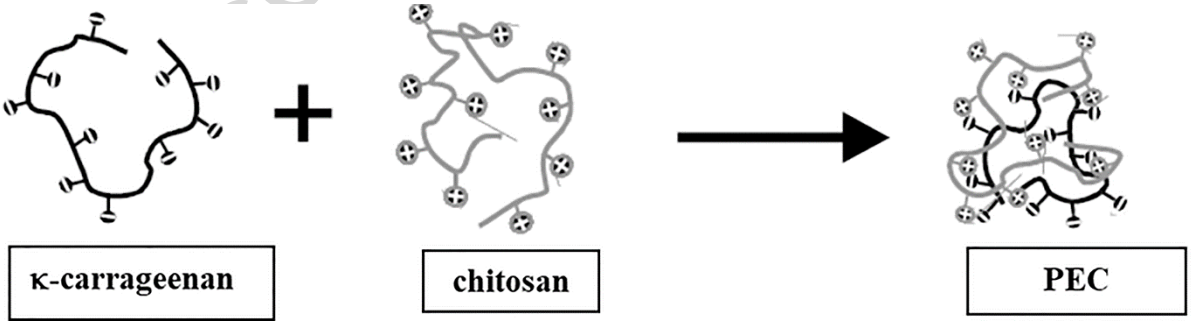


Figure 2: Schematic drawing of complexation of oppositely charged polysaccharides

Many polysaccharide based PECs have been used as capsules for the encapsulation of drugs to enhance their controlled and prolonged release. For chitosan- k-carrageenan PEC capsules, it

has been shown that the theophylline release rate decreases when the concentration of chitosan increases, the capsule diameter increasing accordingly [3]. Better results have been obtained with chitosan-alginate PEC capsules, in the case of diltiazem clorhydrate encapsulation, because carrageenan promotes the entry of water into the matrix and then desintegration instead of swelling [4]. Upon cross-linking with glutaraldehyde, prolonged drug release was obtained: over 24 hours for sodium diclofenac [5]. Glucose oxidase enzyme (isolectric point of 4.2) was negatively charged when the pH > 4.2 and could form a complex with chitosan through electrostatic interactions. The resulting cationic GOD-loaded chitosan was then combined with carrageenan to form a complex via electrostatic interaction. Higher encapsulation efficiency of GOD in chitosan/carrageenan complexes was observed as compared to a single polymer. The efficiency was doubled in polymer blends, which exhibited greater ability to encapsulate protein compared to a single polymer. At a charge ratio of 3, chitosan/κ-carrageenan complexes showed the highest encapsulation efficiency (79%) [6]. The ability of chitosan-κ-carrageenan PEC capsules to protect protein integrity under acidic conditions make them a promising drug delivery system for the oral administration of peptides and proteins.

There have been no studies reported yet on the use of a chitosan–carrageenan polyelectrolyte complex in biosensor application. Chitosan based electrochemical biosensors use chitosan as an electrode coating in order to limit interferences, glucose oxidase being grafted on top of the chitosan layer [7, 8].

Hence, the objective of this work is to evaluate the possibility of using the chitosan/κ-carrageenan polyelectrolyte complex for the conception of a voltammetric glucose biosensor, glucose oxidase being encapsulated in this PEC, using a simple coacervation process. This method is a mild process in a pure aqueous environment.

2. Experimental

2.1. Reagents

Chitosan (average MW= 45 kDa with a degree of acetylation >75%) was obtained from Sigma Aldrich. κ-carrageenan was extracted from red algae of the genus *Halurus*.SP, 4-aminothiolphenol (4-ATP, 97%), glutaraldehyde (GA, 25 wt% aqueous solution), acetic acid (99.8 %), N-(2-Hydroxyethyl) piperazine-N′-(2-ethanesulfonic acid) (HEPES), glucose and glucose oxidase from *Aspergillus niger* (GOx, type X-S, 100–250 kU/g solid) were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). All reagents were used without further purification. Aqueous solutions were prepared using ultrapure water from a MilliQ purification

system. Phosphate saline buffer solutions (PBS, pH 7.4, 0.1 M) were prepared from monopotassic and dipotassic phosphate salts, sodium and potassium chlorides from Sigma Aldrich and adjusted to pH 7.4.

2.2. Extraction of κ-carrageenan

Red algae of the genus *Halurus Sp*. were harvested, in the spring, from the coast of Bizerte (Tunisia) at a depth of 3 meters. After cleaning with distilled water to remove solid waste (sand), the algae were dried in an oven at 40 ° C until they formed a constant mass.

The dried and blended algae were treated in deionized water (Milli Q process) (1/30 w/v) at 90°C for 2 hrs. After extracting the carrageenan, hot filtration was carried out to remove algae residues. The filtrate was placed at 4°C for 4 hrs to obtain a gel [9]. Carrageenan was isolated by filtration, washed with cold water (4°C), frozen and lyophilized.

Carrageenan powder (100 g) was mixed and stirred in 800 mL of 0.1 M KCl solution and then the mixture was centrifuged at 3000 rpm for 30 min. λ-carrageenan was in the surpernatant whereas potassium κ-carrageenan was recovered as a precipitate. This latter was solubilized in a 0.5 M Na_2CO_3 aqueous solution for 1 hr and then dialyzed, frozen and lyophilized.

2.3. Instrumentation

Size exclusion chromatography (SEC) was carried out using an Agilent Technologies system equipped with a guard column (Agilent Polymer Laboratories PolarGel M (50 x 7.5 mm)) and with one column mounted in series (Agilent Polymer Laboratories PolarGel M (300 x 7.5 mm)). A refractive index detector (Waters 2414) allowed on-line detection. 5 µL of κ-carrageenan solution in water, at a concentration of 1 % (w/w), were injected. Elution was achieved at a flow rate of 1 mL/min with 0.05M and 0.1M $(NH_4)_2 CO_3$ aqueous solution at 30°C. Molecular weight calculations were done using Polymer Laboratories Cirrus software based on a calibration curve obtained with polysaccharide standards.

Scanning electron microscopy (SEM) images were obtained using a VEGA TESCAN SEM.

Infrared spectra were recorded at room temperature using a Nicolet Continuum microscope coupled with Nexus infrared spectroscopy in specular reflectance mode equipped with an MCT detector. Recordings were obtained with a resolution of 4 cm^{-1}, a spectral width between 690 and 4000 cm^{-1} and signal processing through Happgenzel apodization. (256 scans).

Electrochemical measurements by cyclic voltammetry (CV) and square wave voltammetry (SWV) were all performed at room temperature. A Voltalab 80 model PGZ 402 analyzer instrument (Hach Lange, France) controlled with Voltamaster 4.0 software, connected to a 5 mL electrochemical cell equipped with a conventional three electrode configuration were employed. A platinum plate was used as the auxiliary electrode (active surface: 0.19 cm^2), while a saturated calomel electrode (SCE) served as reference, and the GOx (CHIT/CAR) modified gold plate as the working electrode (active surface: 0.07 cm^2).

The different step for the elaboration of GOx (CHIT/CAR) modified electrodes were characterized by CV, in 0.1 M phosphate buffer solution (PBS) pH 7.4 containing 10 mM $Fe[(CN)]^{3+/4+}$ couple. The potential was cycled from 0 mV and +800 mV with a scan rate of 100mV/s until several consecutive curves were superimposed.

Electrochemical detection of glucose was performed through cyclic voltammetry and square wave voltammetry. Square wave voltammetry (SWV) was carried out between 0 mV and +800 mV with a pulse amplitude of 1 mV, a pulse width of 0.02 ms, and a potential increment of 10 mV.

2.4. Preparation of polysaccharide complex /GOx mixture

At a charge ratio of 3, chitosan/κ-carrageenan complexes showed the highest encapsulation efficiency for glucose oxidase (79%) [6]. The chitosan solution was prepared at 3 mg/2 ml (1.4 mL of HEPES buffer solution at pH 7.4 completed with 0.6 mL of acetic acid), the final pH value being 2. κ-Carrageenan solution (1 mg/2 ml of water) was then slowly added to the chitosan solution and thoroughly mixed to allow that the coacervation process takes place. Another 15 min of stabilization at room temperature was then allowed. Glucose oxidase (GOx) (10 mg/mL) was added to PEC solution. The mixture was allowed to stabilize at room temperature for 15 min, Thereafter the final mixture was kept for 24 hrs at 4°C.

2.5. Elaboration of the GOx (CHIT/CAR) film on gold electrodes

Gold substrates were provided by the French RENATECH network (LAAS, CNRS Toulouse). They were fabricated using standard silicon technologies. (100)-oriented, P-type (3–5 Ω.cm) silicon wafers were thermally oxidized to grow an 800 nm-thick field oxide. Then, a 30 nm-thick titanium layer followed by a 300 nm thick gold top layer were deposited by evaporation under vacuum.

The gold electrodes were 1.2×1.2 cm^2 square plates. Prior to their modification, the electrode surfaces were cleaned in acetone for 5 min, then in ultra-pure water, and finally dipped in a piranha solution (H_2SO_4:H_2O_2 = 3:1 v/v) for 5 min, then cleaned in ultra-pure water, with final drying under a nitrogen flow.

Subsequently, the electrodes were incubated in a 10 mM ethanolic solution of 4-ATP for 12 hrs in order to form an anchoring mono layer at the electrode surface. Then, the electrodes were rinsed in ethanol. After that, 10 µl of the homogenized mixture [GOx (CHIT/CAR)] was dropped onto the gold plate surface. The biosensor was then placed in saturated glutraldehyde vapor (cross-linker) for 20 min and was stored at 4°C for 24 h. All the modified electrodes were immersed into PBS pH 7.4 to examine their water stability. The preparation procedure is summarized in Figure 3.

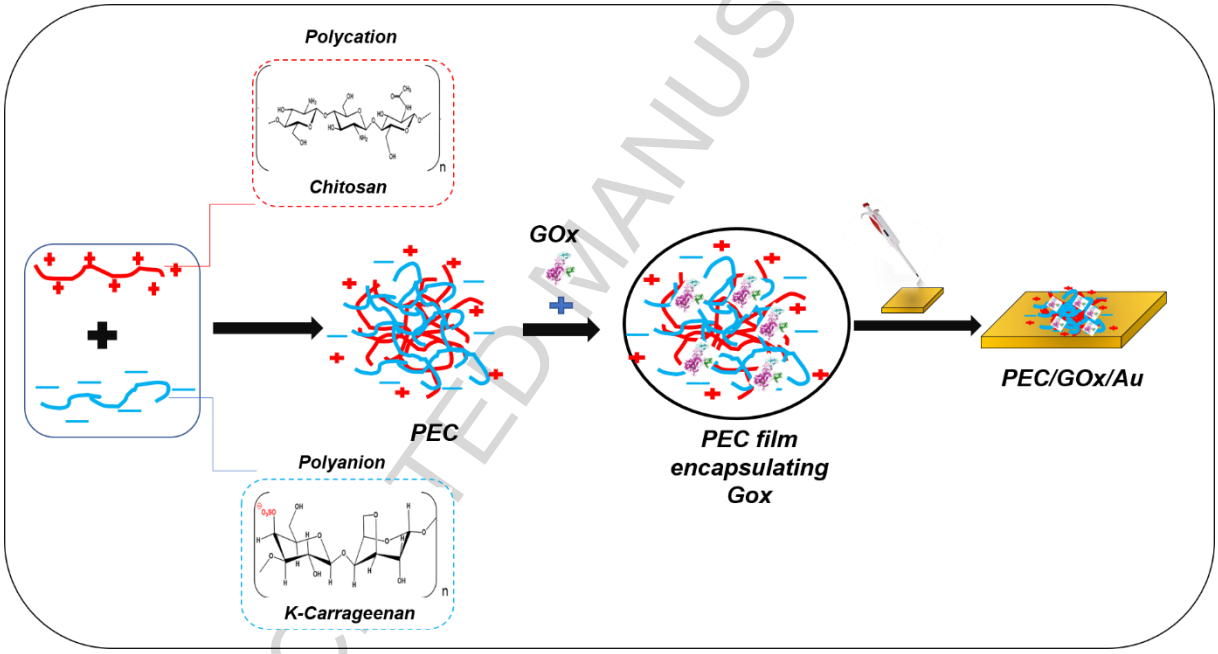


Figure 3: Schematic illustration the different steps involved in the fabrication of the GOx (CHIT/CAR) film on gold electrodes

3. Results and Discussion

3.1. Characterization of extracted carrageenan

After extraction and purification, samples of κ-carrageenan were characterized by SEC. The molecular weight is 750 kDa with a polydispersity index of 1.7. The FTIR spectrum (Figure 4) shows the characteristic bands that confirm the presence of κ- carrageenan [10]:

- 1230 cm^{-1}: Ester sulphate
- 1010-1080 cm^{-1}: Glycosidic link

- 840–850 cm^{-1}: D-galactose-4-sulphate
- No band was detected at 800–805cm^{-1} attributed to 3,6-anhydro-D-galactose-2-sulphate and at 810–820 cm^{-1} attributed to D-galactose-6-sulphate unit, absent in κ-carrageenan

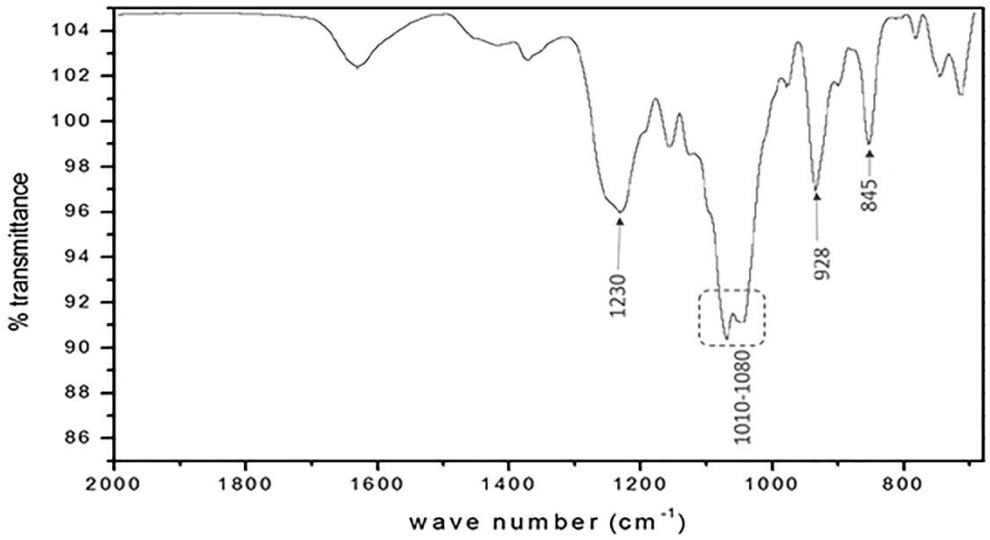


Figure 4: Infrared spectrum of the extracted κ-carrageenan.

3.2. Conductometric monitoring of the formation of the PEC complex and of the encapsulation of GOx.

Conductivities of 25 mM NaCl solutions (pH 2) without and with pure chitosan (1.5 mg/mL) and with chitosan/k-carrageenan (ratio 3) were measured, as reported in Figure 5. The conductivity decreased of 7% when chitosan was added, which shows that when chitosan is solubilized, sodium ions are electrostatically attracted on chitosan chains, decreasing the conductivity of the solution. After addidtion of κ-carrageenan at the stoechiometry, the PEC complex is formed, at the stoechiometry, the total charge is zero and all the ions are liberated, the conductivity of the solution comes back to the inital value.

When glucose oxidase is added, (at this pH value, it is negatively charged) the conductivity of the solution decreases of 7%, due to the electrostic attraction of sodium ions by glucose oxidase. This point shows the glucose oxidase was adsorbed on the PEC complex, without any other change. If the PEC complex would be dissociated, the decrease of the conductivity would be at a larger amplitude, due to the attraction of sodium ions and of chloride ions by liberated chitosan and κ-carrageenan.

Theses conductometric results show that glucose oxidase is dispersed in suspension of PEC globules. After deposition on the electrod surface and evaporation of free water, PEC globules will behave as a glue and will protect protein intergrity.

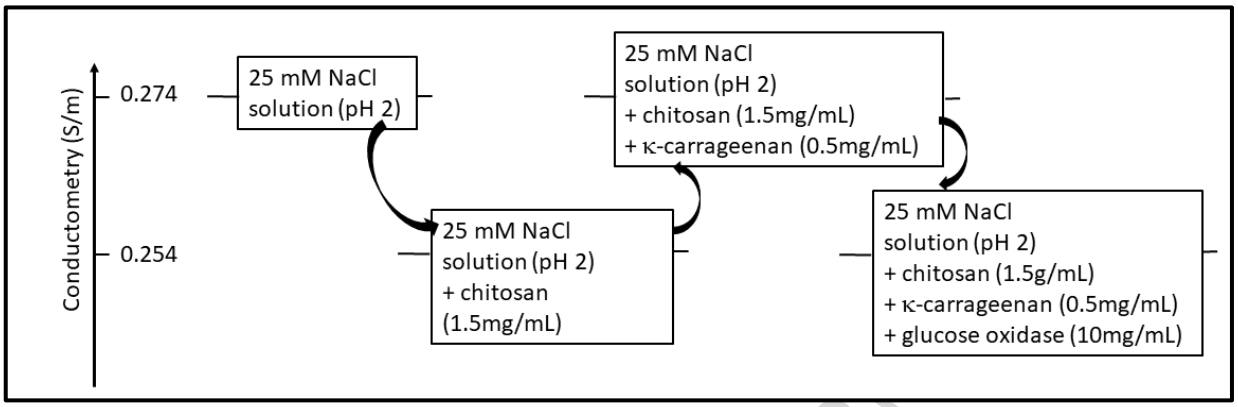


Figure 5: Conductometric monitoring of the formation of the PEC complex and of the encapsulation of GOx.

3.3. Spectroscopic and morphological characterizations of the GOx (CHIT/CAR) film

The formation of the GOx (CHIT/CAR) film on the electrode surface was further confirmed via FTIR spectroscopy. Figure 6 shows the FTIR spectrum of the CHIT/CAR film (Fig.6.a) and that of the GOx (CHIT/CAR) film (Fig.6.b).

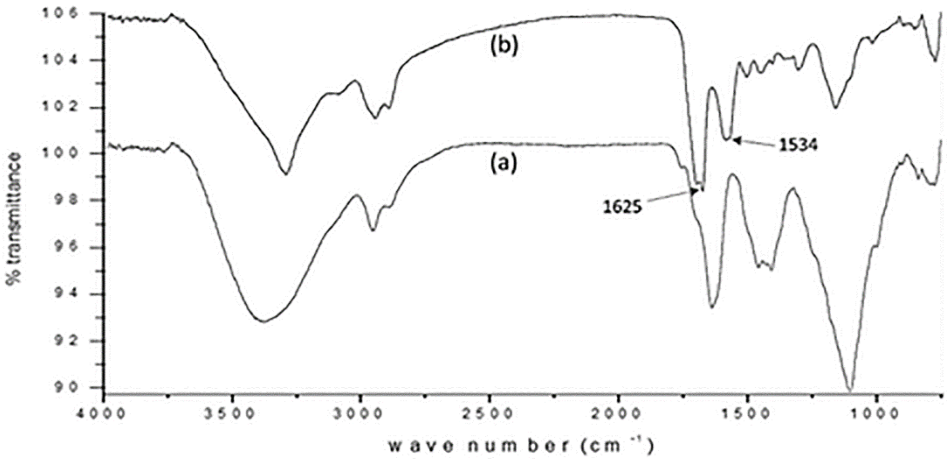


Figure 6: FTIR spectra of the (CHIT/CAR) film before (a) and after (b) encapsulation of the GOx enzyme

The presence of the encapsulated GOx in the chitosan/ κ-carrageenan film is confirmed by the appearance of the bands of the secondary amide characteristic of the enzyme: Amide N-H deformation and C-N stretching vibrations (Amide II band): 1532 cm^{-1} and νC=O: amide C=O stretching vibrations (Amide I band): 1625 cm^{-1}.

The morphologies of the chitosan film encapsulating glucose oxidase (Figure 7a) and of the chitosan/ κ-carrageenan film encapsulating glucose oxidase (Figure 7b) were observed using

SEM. Chitosan film presents an irregular morphology and a high roughness compared to those of the chitosan/ κ-carrageenan film. This is due to the higher plasticity [11] and a higher hydrophobicity [2] of the CHIT/CAR PEC which presents a higher affinity for the SAM layer on the gold surface and so higher adhesion. Moreover the CHIT/CAR PEC film composed of globules is compact and act as a glue for protecting GOx integrity.

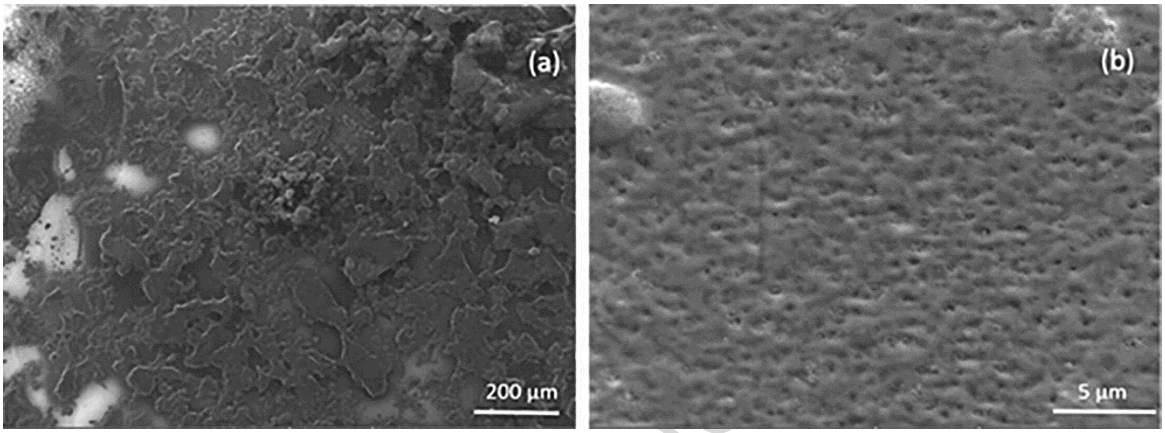


Figure 7: Representative SEM image of (a) GOx (CHIT) film on gold, (b) GOx (CHIT/ CAR) on gold

3.4. Electrochemical characterization of the CHIT/CAR film on gold electrode

Study of charge transfer rate

Each step of the biosensor construction was characterized using a $Fe[(CN)]^{3-/4-}$ couple as redox probe. The separation between anodic and cathodic peak potentials (ΔEp= Epa-Epc) as well as the intensity of the peaks (Ipa and Ipc) are correlated to the electron transfer properties of the electrode. As seen in Figure 8, both oxidation and reduction peaks of the $Fe(CN)_6^{3-}$/ $Fe(CN)_6^{4-}$ couple are clearly detected before the bare gold modification with 4-ATP. After the modification of the gold electrode surface with 4-ATP SAM layer a large increase of ΔEp of 600 mV is observed. When a layer of chitosan is deposited on the SAM, the ΔEp decreases to a value of 361 mV whereas when a layer of carrageenan is deposited on the SAM, the ΔEp decreases by a value of 235 mV. When a layer of CHIT/CAR complex is deposited on the SAM, the observed value of ΔEp is 307 mV. These values show that the presence of carrageenan favors the electron transfer through the film and the CHIT/CAR complex film presents a better charge transfer rate than the pure chitosan film. Nevertheless, the intensities of the anodic and cathodic peaks are lower than those measured with bare gold. By increasing the scan rate (data not shown), the anodic peaks shifted towards more positive values while the cathodic peaks shifted towards more negative potential values,

demonstrating that a quasi-reversible process was taking place. The plot of the anodic peak current at 276 mV versus the square root of the scan rate (data not shown) was a perfect straight line with a correlation coefficient of 0.99, which means that the mass transfer towards the electrode surface is diffusion controlled.

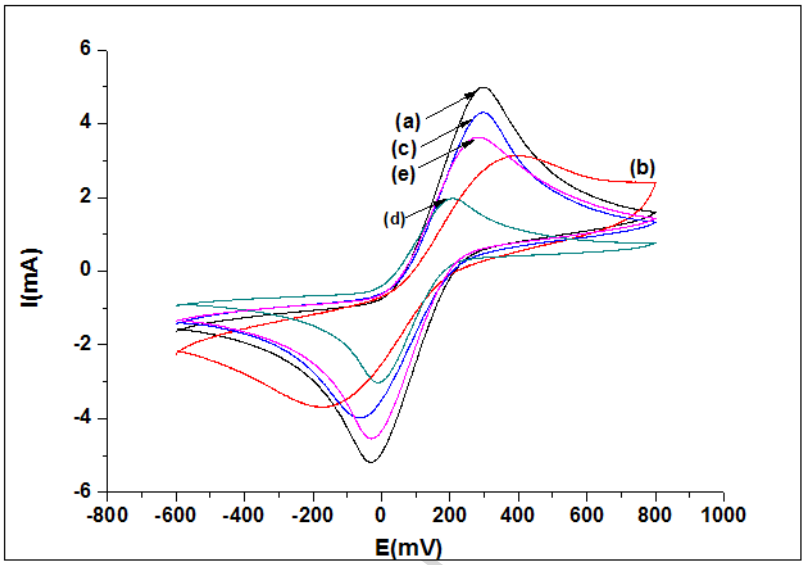


Figure 8: Cyclic voltammograms of modified gold electrodes **(a)** bare Au, **(b)** Au/4-ATP (c) Au/4-ATP/CHIT **(d)** Au/4-ATP/CAR **(e)** Au/4-ATP/CHIT/CAR. Supporting electrolyte:10 mM $[Fe[(CN)]^{3-/4-}$ in PBS solution (0.1M, pH 7.4). Scan rate 100 mV/s

Study of glucose detection with GOx encapsulated in chitosan and in CHIT/CAR films

The CV curve in the presence of 2 mM glucose solution, obtained with GOx encapsulated in chitosan, is curve 9a. The redox couple corresponding to hydrogen peroxide presents an oxidation peak at 492 mV, (Ia = 51 µA) and a weak reduction peak at 425 mV (Ic = 20 µA), with a ΔEp = 67 mV.

The CV curve in the presence of 2 mM glucose solution, obtained with GOx encapsulated in CHIT/CAR PEC, is curve 9b. The redox couple corresponding to hydrogen peroxide presents an oxidation peak at 495 mV, (Ia = 65 µA) and a weak reduction peak at 440 mV (Ic = 22 µA), with a ΔEp = 55 mV. Then a higher reversibility is observed in presence of CHIT/CAR PEC and also higher intensity.

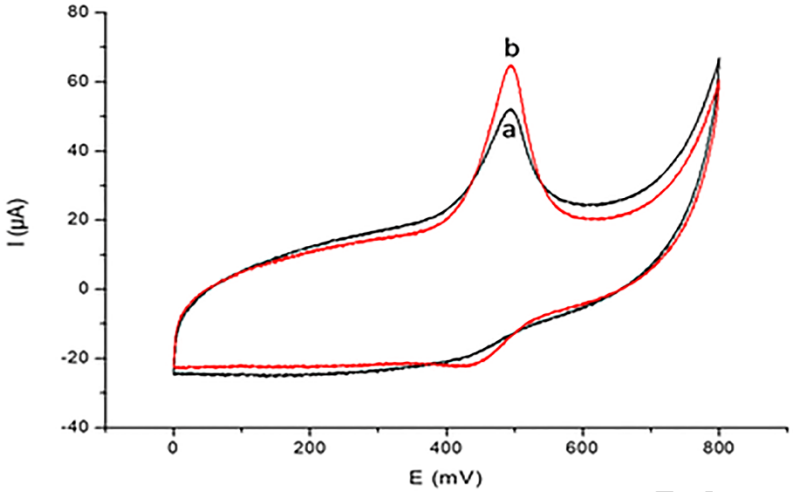


Figure 9: CVs of the detection of glucose at a concentration of 2 mM by the Au electrode modified by **(a)** CHIT and **(b)** CHIT/CAR, CV performed at 100 mV/s in PBS solution (0.1M, pH 7.4)

3.4. Analytical performance of the glucose biosensor

The calibration of glucose biosensors obtained after glucose oxidase encapsulation in a chitosan film (CHIT glucose biosensors) and after encapsulation of glucose oxidase in a chitosan-κ-carrageenan PEC complex (CHIT/CAR glucose biosensor) was performed and compared.

The variation of the SWV anodic peak obtained with the CHIT glucose biosensors with increasing concentrations of glucose is reported in Figure 10. The SWV anodic peak is obtained at 460 mV and the peak potential value is shifted towards positive values when the concentration of glucose increases. The calibration curve is presented in Figure 11. The linear range is between 200 µM to 10 mM. The detection limit is calculated according to the following expression: $2\sigma/|S|$, σ being the noise from the blank, and $|S|$ the sensitivity, slope of the calibration curve; the limit of detection (LOD) is equal to 63 µM.

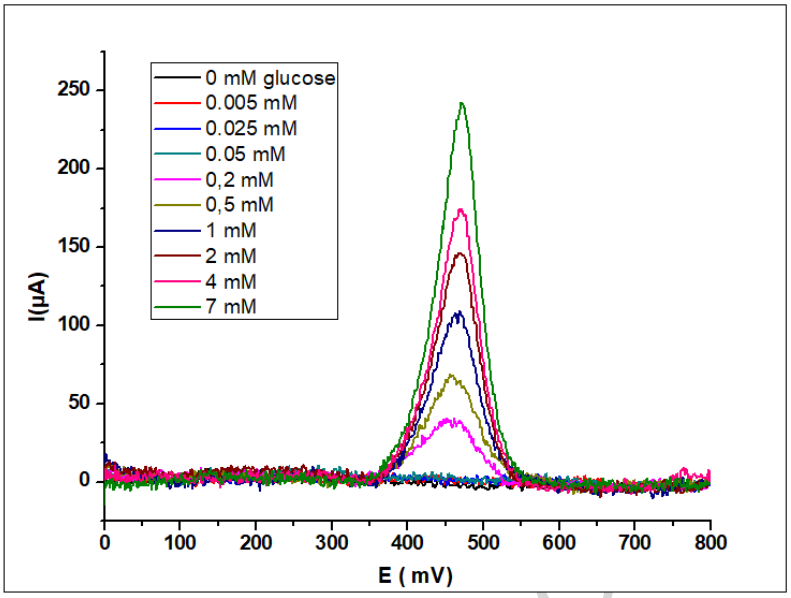


Figure 10: SWV anodic peak obtained for Au/CHIT/GOx modified electrode upon injection of increasing concentrations of glucose (0 -7 mM), in PBS solution (0.1M, pH 7.4)

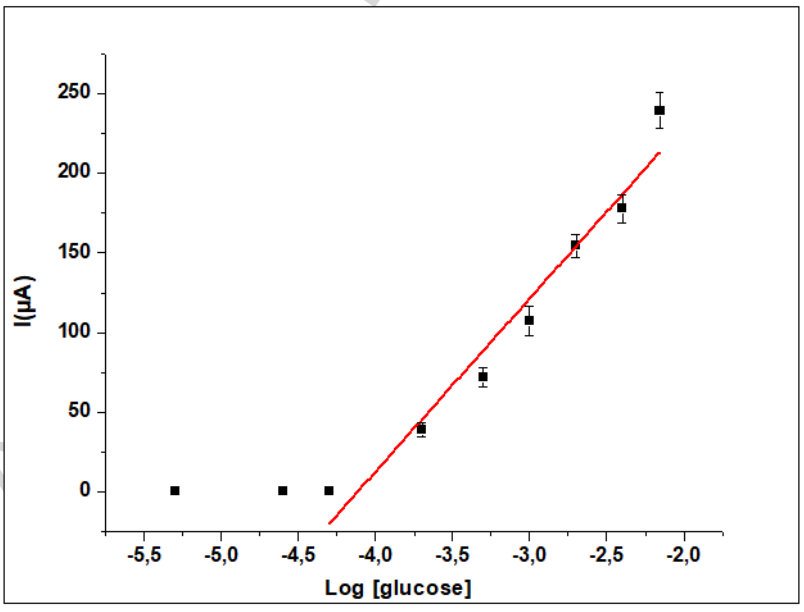


Figure 11: Calibration curve obtained for Au/CHIT modified electrode upon injection of increasing concentrations of glucose (0 -7 mM), in PBS solution (0.1M, pH 7.4).

For the GOx (CHIT/CAR) based biosensor, the SWV anodic peak is also obtained at 460 mV and the peak potential value is also shifted towards positive values when the concentration of glucose increases (cf Figure 12). The calibration curve is presented in Figure 13. The linear range is between 10 µM to 7 mM. The detection limit is 5 µM. A lower detection limit is obtained for the GOx (CHIT/CAR) based biosensor whereas both sensors present the same sensitivity.

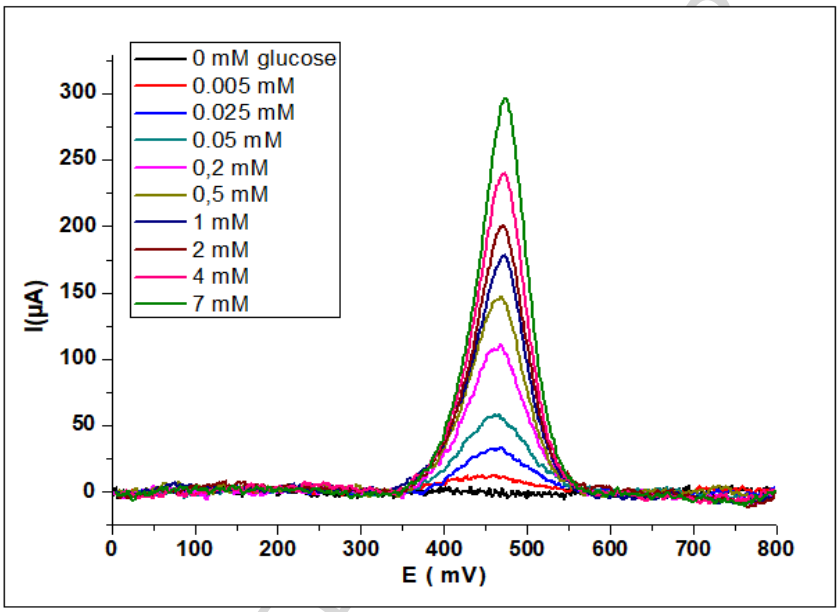


Figure 12: SWV obtained for Au/CHIT-CAR/Gox modified electrode upon injection of increasing concentrations of glucose (0 - 7 mM), in PBS solution (0.1M, pH 7.4)

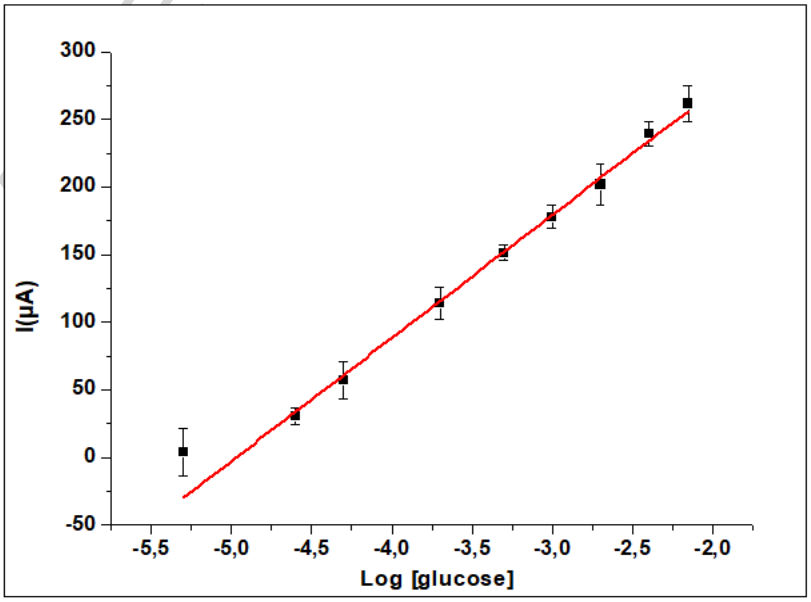


Figure 13: Calibration curve of the Au/CHIT-CAR biosensor (in PBS 0.1, pH 7.4)

The analytical performances of the GO_X (CHIT) and GOx (CHIT/CAR) based biosensors are compared to those of recently published chitosan or carrageenan based glucose biosensors. (cf Table). The detection limit of the GOx (CHIT/CAR) based biosensor is in the lower range of the detection limits of the other amperometric/voltammetric biosensors and the dynamic range is among the larger ranges.

Electrochemical Technique	Hybrid Nanomaterials/polysaccharide/GOx	Detection Limit	Dynamic range	Reference
Voltammetry	Chitosan/PAA/GOx	10 µM	0.05 – 15 mM	[7]
Voltammetry (DPV)	K-carrageenan/PANI/GOx		1 – 15 mM	[12]
Amperometry	AgNWs/chitosan/GOx/		1 – 15 mM	[13]
Amperometry	GRCO/AC/PtNP/chitosan/GOx/Nafion	2 µM	2 µM – 10 mM	[14]
Amperometry	GO_X /RGO/ZnONPs/AgNPs/chitosan/ GC	10.6 µM	0.1mM – 12.0 mM	[15]
Amperometry	Chitosan/PPy/AuNPs/ITO	3.1 µM	0 – 3.2 mM	[16]
Voltammetry	PPy/Nafion/fMWCNTs/chisan/GOx	5 µM	0 - 4.7 mM	[17]
Amperometry	GOx-ImAS-CS/RGO-PtNP/Au	0.02 µM	2 µM – 5.5 mM	[18]
Amperometry	Chitosan/GOx	7.4 µM	0.2 mM – 9 mM	[19]
Amperometry	GOx/ Chitosan-Covered Pd Pt Core-Shell Nanotubes		1 – 6 mM	[20]
Voltammetry	GOx/MCNT/chitosan	50 µM	0.2 – 1.2 mM	[21]
Voltammetry	PPy/Nafion/chisan/GOx	4.3 µM	0-2 mM	[22]
Voltammetry	Chitosan/TiO2NTAs	70 µM	0.3 to 1.5 mM	[23]
Voltammetry	CS/GOx-poly(5-HT)-PdNPs/Pd-plate/Au	200 µM	2 mM – 6.6 mM	[24]
Voltammetry	PEDOT/Chitosan/PSS	41 µM	0.1 – 1.4 mM	[25]
Voltammetry (SWV)	chitosan	63 µM	200 µM – 7mM	This work
Voltammetry (SWV)	Chitosan/K-carrageenan	5 µM	10 µM – 7 mM	This work

Table: Comparison of the analytical performances of the GO_X (CHIT) and GOx (CHIT/CAR) based biosensors to those of the recently published chitosan or carrageenan based glucose biosensors

Reproductibility and shelf lifetime of the GOx (CHIT/CAR) based biosensor

The stability of the biosensor was studied for three weeks. In Figure 14 is reported the SWV peak maximum for the detection of 0.2 mM glucose concentration at different days, after being kept at 4 °C in refrigerator when not in use. The response remain stable during this period, with a RSD of 5%.

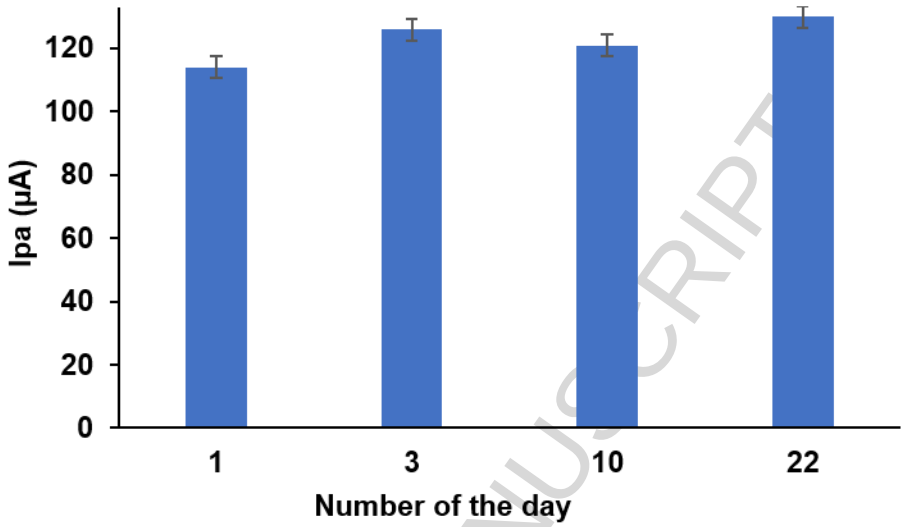


Figure 14: SWV peak maximum for the detection of 0.2 mM glucose concentration at different days

The relative standard deviation (RSD) obtained, for intra sensor reproducibility is 6%.

Selectivity of the GOx (CHIT/CAR) based biosensor and application in spiked saliva samples

In order to demonstrate the selectivity of the glucose biosensor, the latter was tested for the detection of 0.2 mM of glucose, in the presence of different analytes: ascorbic acid (Figure 15a), uric acid (Figure 15b), urea (Figure 15c). The percentages of variation of the peak maximum for the detection of 0.2 mM glucose concentration, in the presence of the interfering substances are respectively 96.5 ± 7.5 for ascorbic acid, 102.7 ± 2.8 for uric acid and 101 ± 5 for urea. These results show that the encapsulation of GOx in PEC limit the interference of the electroactive species.

To study the practical performance of the proposedcbiosensor, it was employed to determine trace amounts of glucose in spiked saliva samples. For the analysis, saliva samples were spiked with 50 µM of glucose and analyzed after dilution (10%) in PBS solution. The recoveries was 105 %, validating the potential utility of the present biosensor for glucose detection in biological samples such as saliva samples.

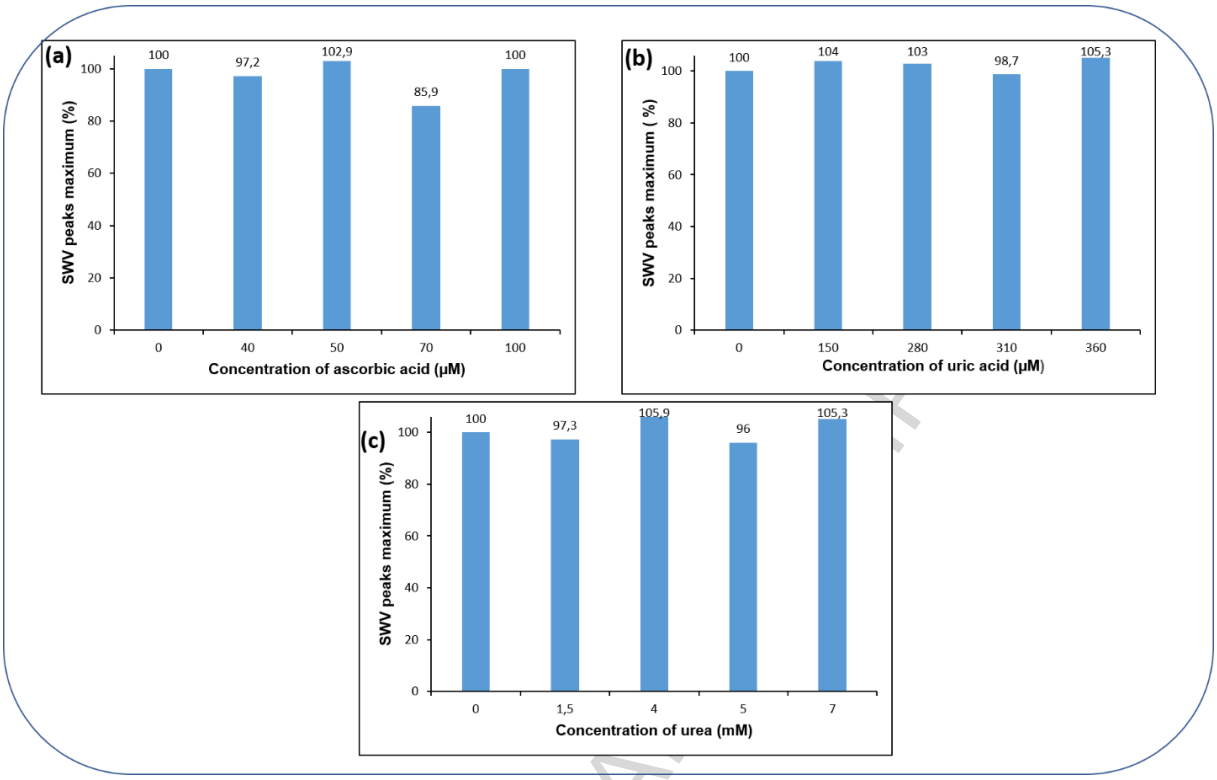


Figure 15: SWV peak maximum for the detection of 0.2 mM glucose concentration in presence of different concentration of possibly interfering substances (a) ascorbic acid (b) uric acid (c) urea

4. Conclusion

We have constructed a simple but effective biosensor for glucose detection. As a key element of the sensor, a polyelectrolyte complex of κ-carrageenan and chitosan has been developed in order to provide an effective encapsulation of the glucose oxidase enzyme. The simple preparation procedure adopted resulted in a glucose sensor with a broad linear range and a low detection limit. It also exhibits an excellent reproducibility, repeatability, stability and selectivity and applicability in saliva samples. The analytical performance of this type of biosensor is very competitive, without using any metallic nanoparticles. The excellent biodegradable properties of this biosensor are then of high interest for the circular economy.

References

[1] J. Venkatesan, B. Lowe, S. Anil, P. Manivasagan, A. A Al Kheraif, K.H. Kang, S.K. Kim, Starch/Stärke **2015**, *67*, 381.

[2] Y. A. Shchipunov and I. V. Postnova, Composite Interfaces **2012**, *16*, 251.

[3] H. Tomida, C. Nakamura, S. Kiryu , **1994**, 42, 979.

[4] C. Tapia, Z. Escobar, E. Costa, J. Sapag-Hagar, F. Valenzuela, C. Basualto, M.N. Gai, M. Yazdami-Pedram, European Journal of Pharmaceutics and Biopharmaceutics **2004**, *57*, 65.

[5] P. Piyakulawat, N.Praphairaksit, N. Chantarasiri,N. Muangsin, American Association of Pharmaceutical Scientists **2007**, *8*, 97.

[6] A.V.Briones and T.Sato, Reactive & Functional Polymers **2010**, *70*, 19.

[7] M Yang, Y Yang, B Liu, G Shen and R Yu , Sensors and Actuators B:chemical **2004**, *101*, 269.

[8] H. Dai, X. Wu, H. Xu, Y. Wang, Y. Chi and G. Chen, Electrochimica Acta **2009**, *54*, 4582.

[9] L. Youssouf, L. Lallemand, P. Giraud, F. Soulé, A. Bhaw-Luximon, O. Meilhac, C. Lefèbvre D'Hellencourt, D. Jhurry, J. Couprie, Carbohydrate Polymers **2017**, *166*, 55.

[10] D. Jhurry, A. Bhaw-Luximon, T. Mardamootoo, A. Ramanjooloo, Macromolecular Symposia **2006**, *231*, 16.

[11] M. Shahbazi, G. Rajabzadeh, R. Ettelaie, A. Rafe, Carbohydrate Polymers **2016**, *142*, 167.

[12] D. Zhu, H. Nakamura, H. Zhu, C. Xu, M. Matsuo, Synthetic Metals **2015**, *202*, 133.

[13] S. Kumar-Krishnan, S. Chakaravarthy, A. Hernandez-Rangel, E. Prokhorov, G. Luna-Barcenas, R. Esparza and M. Meyyappan, RSC Adv. **2016**, *6*, 20102.

[14] M. F. Hossain and J. Y. Park, Scientific Reports **2016**, *6*, 21009

[15] Z. Li, L. Sheng, A. Meng, C. Xie, K. Zhao, Microchimica Acta **2016**, *183*, 1625

[16] A. Sharma, A. Kumar, Synthetic Metals **2016**, *220*, 551

[17] B. K. Shrestha, R. Ahmad, H. M. Mousa, I.G. Kim, J. I. Kim, M. Prasad Neupane, C. H. Park, C. S. Kim, J. Coll. Interf. Sci. **2016**, *482*, 39.

[18] F. Wu, T. Huang, Y. Hu, X. Yang, Q. Xie, J. Electroanal. Chem. **2016**, *781*, 296.

[19] H. Zare, G. D. Najafpour, M. Jahanshahi, M. Rahimnejad, M. Rezvani, Romanian Biotechnological Letters, **2017**, *22*, 12611.

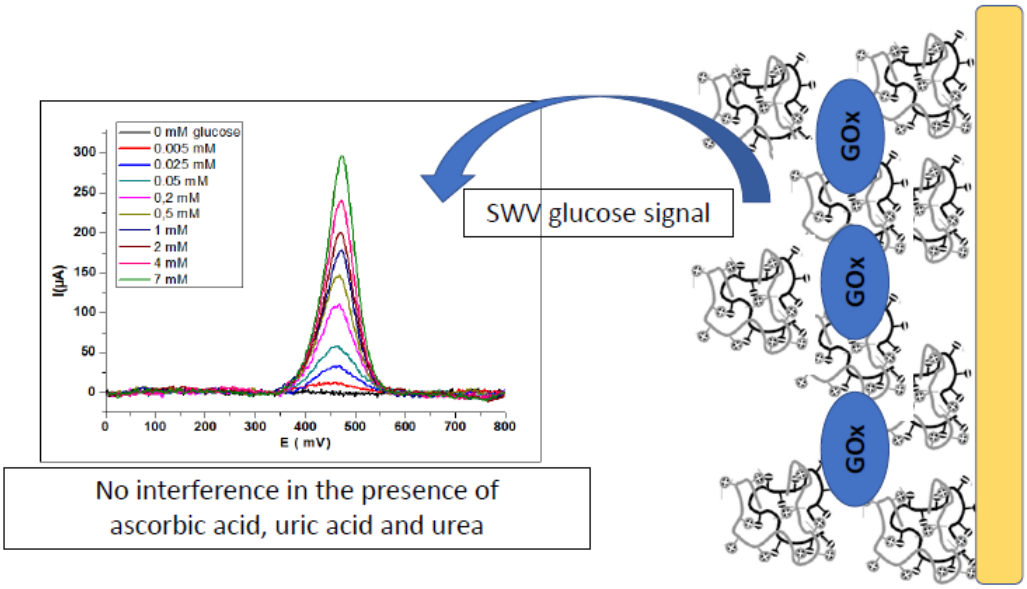
[20] S. Kumar-Krishnan, E. Prokhorov, D. Bahena, R. Esparza, M. Meyyappan, ACS Omega **2017**, *2*, 1896.

[21] E. Uwimbabazi, M.R. Mukasekuru, X. Sun, Xiulan, Food Analytical Methods, 2017, 10, 2667.

[22] B. Wang, B. Koo, H. G. Monbouquette, Electroanalysis **2017**, *29*, 2300

[23] M. Artigues, J. Abella, S. Colominas, Sensors **2017**, *17*, 2620.

[24] T. Huang, Z. Liu, Y. Li, Y. Li, L. Chao, C. Chen, Y. Tan, Q. Xie, S. Yao, Y. Wu, Anal. Chim. Acta **2018**, *1013*, 26.



Graphical abstract

Highlights

Glucose oxidase is encapsulated in a chitosan/κ-carrageenan polyelectrolyte complex

A more sensitive voltammetric detection of glucose is obtained compared to that based on chitosan film.

The biosensor exhibits a wide linearity range from 5 µM to 7 mM glucose.

The biosensor exhibits a detection limit of 5 µM glucose.

Excellent selectivity against ascorbic acid, uric acid and urea is observed.