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Biosensor characterization from *Cratylia mollis* seed lectin (Cramoll)–MOF and specific carbohydrate interactions in an electrochemical model

Maryana Hermínio de Carvalho[a,b], Hallysson Douglas Andrade de Araújo[a], Renata Pereira da Silva[b], Maria Tereza dos Santos Correia[a], Katia Cristina Silva de Freitas[b], Sandra Rodrigues de Souza[c], Luana Cassandra Breitenbach Barroso Coelho[a]*

[a] Departamento de Bioquímica, Centro de Biociências, CB, Universidade Federal de Pernambuco, Avenida Prof. Moraes Rego, 1235, Cidade Universitária, 50670-420, Recife-PE, Brazil.

[b] Departamento de Química, Universidade Federal Rural de Pernambuco, UFRPE, Rua Dom Manuel de Medeiros, S/N, Dois Irmãos, Recife-PE, CEP 52171-900, Brazil.

[c] Departamento de Educação, UFRPE, Rua Dom Manuel de Medeiros, S/N, Dois Irmãos, Recife-PE, CEP 52171-900, Brazil.

* Corresponding author: E-mail address: lcbbcoelho@gmail.com (L. C. B. B. Coelho).

Abstract

Biosensors are small devices known for their selectivity, high specificity and sensitivity to the respective analyte, at low concentrations. We developed an electrochemical biosensor using the crystalline polymer MOF- $[Cu_3(BTC)_2H_2O)_2]n$ to characterize *Cratylia mollis* seed lectin (Cramoll) and its interaction with free carbohydrate (glucose) and carbohydrates on the surface of rabbit erythrocytes. The electrochemical potentials presented by the exponential curves that vary from 96 to 142 mV in relation to concentrations of 10 to 20 mM of glucose are decisive for the use of the system containing gold electrode/MOF/Cramoll for the

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characterization of biological models due to its high sensitivity. As well as the kinetic behavior presented in the cyclic voltammograms, with a cathodic current response of 0.000 3 A for a glucose concentration of 15 mM. These results were due to the high specificity of Cramoll under these conditions, promoting stability of surface charges at the Cramoll/electrode interface. This phenomenon facilitates the monitoring of the interaction with free glucose present in the electrolyte medium by potentiometric and amperiometric methods and with carbohydrates present on the surface of rabbit erythrocytes through the potentiometric method. Through scanning electron microscopy (SEM) it was possible to observe Cramoll immobilized on the MOF surface, proving the specificity of the ligand (glucose-lectin) through the morphological lectin changes in this process. This electrochemical model, Cramoll/MOF biosensor, is effective for evaluating free lectin/carbohydrate or in the erythrocyte membrane.

Keywords: Plant lectin; Electrode surface; Metal-Organic Framework - MOF; Immobilization; Cyclic voltammograms.

Introduction

Electrochemical biosensors are sensors that use biological materials, including proteins, cells and DNA, immobilized on suitable polymers or membranes, and connected to a transducer, which monitor the appearance of reagents or products from the reaction of the biological source with the material of interest, converting this biological signal in electrical signal.[1-3] These selective devices with high sensitivity can help provide specific and fast diagnostics with low cost, ease of use and portability.[4]

Lectins are proteins widely distributed in nature, found in animals, plants, bacteria and viruses, have selective binding sites to carbohydrates or glycan portions from glycoconjugates.[5,6] Lectin-carbohydrate interactions become a viable tool for the analysis of

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biological phenomena[7] seeking to observe physiological responses to these systems, such as mitogenic activity and cell-cell interaction.[8,9] These interactions occur due to the existence of different intermolecular forces, including hydrogen bonds, Van der Walls and hydrophobic interactions.[10,11] The Brazilian Caatinga biome is being studied for its biotechnological potential,[12] there we find a plant popularly as camaratu bean, a forage native to the Caatinga biome, rich in shrub legumes, from which we extract the Cramoll lectin from *Cratylia mollis* Mart. has use in biosensors due to its protein relevance in biological systems.[13,14] Cramoll has the properties of healing wounds,[15] repairing skin wounds in diabetics,[16] detecting arbovirus glycoprotein expression,[17] characterizing malignant tumors,[18,19] among other applications.

Considering the methods of analysis of direct electron transfer in lectin-carbohydrate interactions, potentiometric and amperometric evaluations stand out.[14,17] These methods are directly associated with charge transfer on the lectin surface subjected to an electric field in the presence of its ligand and with experimental handling.[20] To investigate the morphology and conformational changes on surfaces, the scanning electron microscope (SEM) technique, use one of the most versatile instruments available for the observation and analysis of microstructural characteristics of solid materials, quickly provides information on the morphology and identification of chemical elements of the sample.[21] The use of new materials can enhance the detection limit, biocompatibility and stability of sensors,[22] among these inputs, the use of the organic metal Framework (MOF) $[Cu_3(BTC)_2H_2O)_2]n$ stands out, consisting of an extensive crystalline network of metallic ions or clusters, coordinated to multidentate organic molecules/ligands, mostly carboxylates, bipyridines, sulfonates and phosphonates culminating in well-defined porous dimensions.[23] MOF can be used in the treatment of effluents with the presence of dyes,[24] as well as for gas adsorption,[25,26]

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antibiotic adsorption,[27] energy conversion,[28] energy storage,[29] as a catalyst,[30,31] and effective in the rapid detection of pesticides to maintain food quality and safety.[32]

The development of systems that can quickly identify and characterize cell surfaces through the specific identification of glycosides has great value for histopathological studies.[15,33,34] Erythrocytes are being used in biosensors with the aim of evaluating lectins in a complex environment by determining the electrochemical potential.[35] Studies show that electrical properties, mainly membrane capacitance or, equivalently, erythrocyte cell membrane permissivity, is altered by the presence of glucose.[36-39] Therefore, this study aimed to develop an electrochemical biosensor immobilizing the Cramoll lectin and to characterize the chemical and physical properties of interactions with carbohydrates and erythrocyte membranes.

Results and discussion

Cramoll-Glucose Electrochemical Potentials

The immobilization of Cramoll in MOF $[Cu_3(BTC)_2H_2O)_2]n$ did not show significant difference in the resting time of 3 h or 24 h at a temperature of 4 ºC, for adsorption of this lectin to the polymer; thus, the time determined for immobilization was 3 h. Although many immobilization technologies have been proposed, the adsorptive storage in metal-organic structures (MOFs) is promising due to high values of surface area, low density, good thermal and chemical stability and mainly due to the functionalization of its pores, which can reach 90% of its volume with specific surface areas of up to more than 7,000 m^2/g.[23,26, 40,41]

We fixed Cramoll adsorbed on the MOF in a metal working electrode that aimed to generate an electrical potential measured in relation to the reference electrode (Ag/AgCl). Platinum and gold metals were chosen for their electrical and mechanical properties, high

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chemical inertness, providing a charge in the anodic working potential range with low electrical resistivity and high signal-to-noise ratio.[42,43]

Initially, to verify the behavior of the Cramoll adsorbed on the MOF on the platinum electrode, cyclic voltammograms were performed with different concentrations of glucose (Fig. 1A) and fructose (Fig. 1B) 20, 40, 60, 80 and 100 mM.

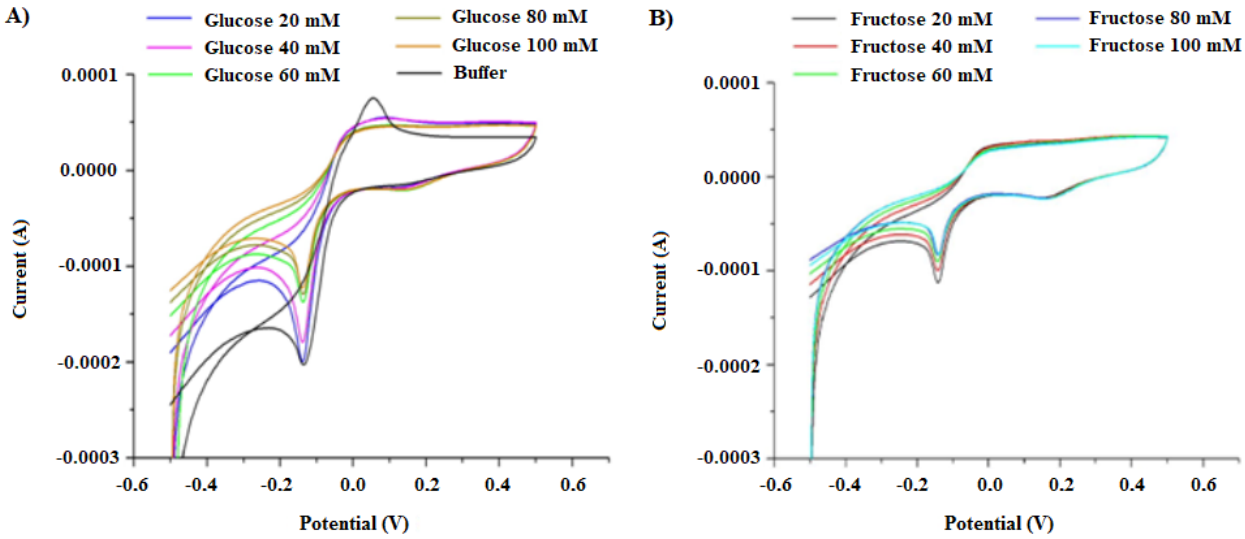

Fig. 1. Cyclic voltammograms with platinum/Cramoll/MOF electrode interacting at different concentrations of (A) glucose and (B) fructose 20 mM, 40 mM, 60 mM, 80 mM and 100 mM.

Comparing the cyclic voltammograms shown in Fig. 1, in the Cramoll/MOF + glucose system, there is an increase in the currents of the cathodic and anodic peaks at concentrations of 20 and 40 mM. It is observed that at concentrations greater than 60 mM of glucose, there are only subtle variations in the currents of these peaks, which indicates the saturation of the Cramoll binding sites with the specific carbohydrate at these concentrations. We can also verify in the Cramoll/MOF + fructose cyclic voltammograms low current generation with small variations in these peaks, revealing a low interaction of Cramoll with this carbohydrate. The results led to the realization of new experiments using platinum/Cramoll/MOF electrode with glucose concentrations of 5, 10 and 15 mM to construct the biosensor (Fig. 2A).

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The biosensor with platinum electrode showed a positive electrochemical potential, ranging from 220 to 240 mV, when the buffer solution was used as support for charge control between the reference electrodes. The addition of glucose to the electrochemical system at all concentrations tested promoted a significant increase in potential (Fig. 2A). Seeking to reduce the amount of MOF and Cramoll lectin used in the platinum electrode, the gold work electrode was built, thus confirming the viability of this last electrode, being observed in Fig. 2B. The addition of glucose to the electrochemical system at the concentrations tested promoted an increase in the electrochemical potential, between 5-10 min (Fig 2B and 2C), and revealed exponencial curves with these times (2B); the electrochemical system was turned off after 30 min. However, at the concentration of 5 mM glucose, it did not show reproducibility in the five repetitions performed, as they were not reported in Fig. 2B. A gradual increase was obtained after exposure to the concentrations of 10 mM (96 - 121 mV), 15 mM (110 - 126 mV) and 20 mM (107 - 142 mV) in time from 5 to 30 min; at 40 mM, the addition of glucose resulted in a non-standard behavior, the characteristic exponential curves did not occur in the times between 5 and 10 min, probably due to the saturation of the interaction sites (Cramoll-Glucose).

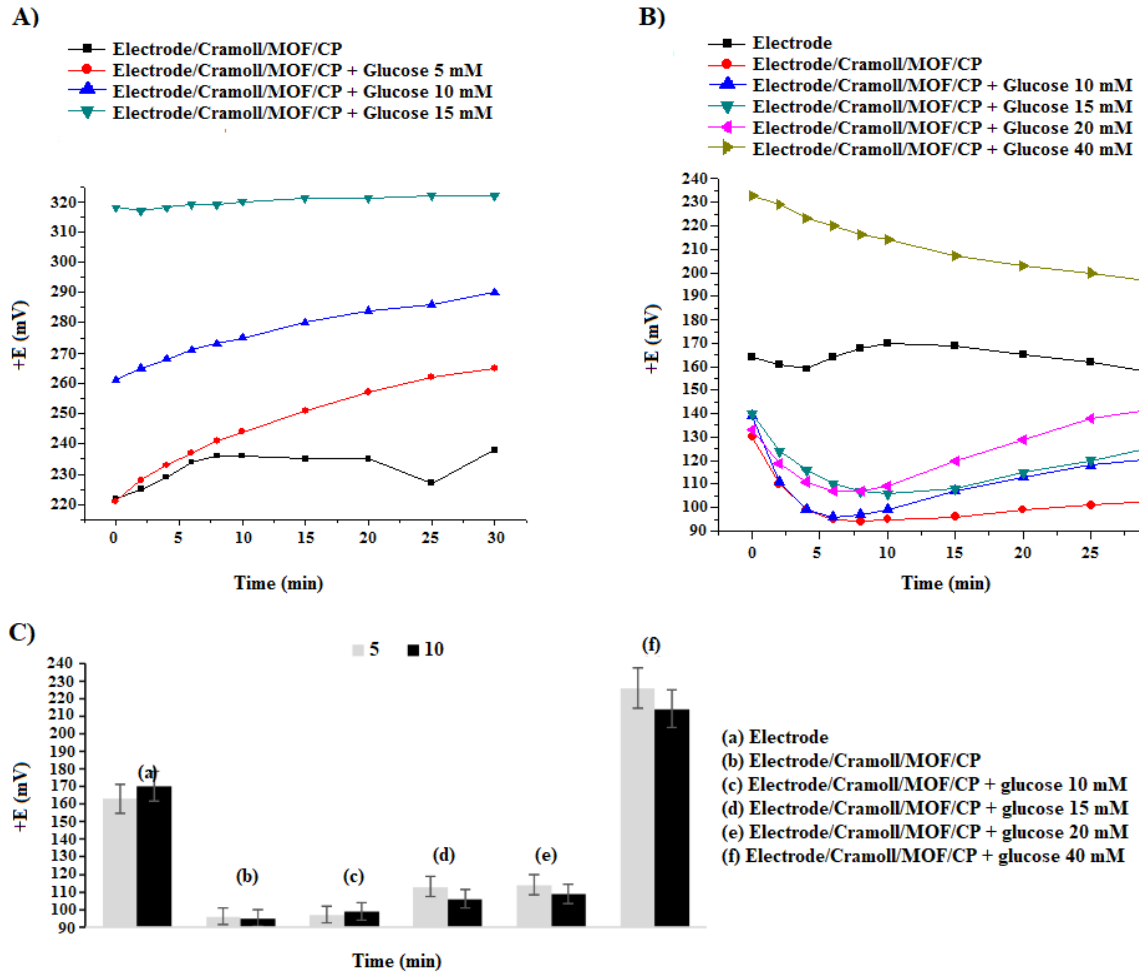

Fig. 2. (A) Electrochemical potentials with a platinum electrode obtained by the Cramoll/MOF interaction with different glucose concentrations; (B) electrochemical potentials obtained with a gold electrode by the Cramoll/MOF interaction with different glucose concentrations; (C) application of bar errors (5%) to experimental results at intervals of 5 and 10 min with a gold electrode, in five repetitions to (a) electrode, (b) electrode/Cramoll/MOF/CP (c) electrode/Cramoll/MOF/CP + glucose 10 mM, (d) electrode/Cramoll/MOF/CP + glucose 15 mM, (e) electrode/Cramoll/MOF/CP + glucose 20 mM, and (f) electrode/Cramoll/MOF/CP + glucose 40 mM.

Cramoll 1,4 is an isoform of *C. mollis* lectin with a classic tertiary structure of legume lectins, having binding sites to methyl-α-D-mannopyranoside carbohydrate, with which they interact by hydrogen bonds, and binding sites to Ca^{2+} and Mn^{2+}. It is a lectin of the glucose/mannose specificity group, with methyl-α-D-mannopyranoside being the monosaccharide for which it has the best affinity.[44] Based on the observations of Souza et al.

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[45,46] the first Cramoll biosensor was built to characterize the binding with glucose showing the possibility of using this lectin in analytical instruments. In this system, the presentation of exponential curves up to 10 min in the determination of the electrochemical potential are characteristics of the interactions of the lectin from seeds of *Canavalia ensiformis*, Concanavalin A (Con A) and Cramoll, with glucose.[45]

The electrochemical potentials obtained due to the interaction of Cramoll adsorbed on the MOF and fixed on platinum and gold electrodes with carbon paste reveal the biological activity of this protein in these modified electrodes [13,20,47]. Copper MOF proved to be a suitable matrix for enzyme immobilization.[48] Its large ordered surface area with remarkable porosity can provide high charge capacity, and its strong affinity can prevent enzyme degradation.[49] It is possible to reaffirm the potential advantages of metal-organic structures in biological applications due to their inherent biodegradability and ability to use biocompatible components.[50]

Potentiometric evaluations for the activity of biomolecules on chemically modified surfaces are indicated for monitoring the interaction with their ligands.[17,43] They measure changes in solution current resulting from biochemical reactions[4,46] with the systems we build. The electrochemical potentials of the Cramoll lectin in the absence and presence of glucose monitor charge transfer electrons between protein and carbohydrate in the electrochemical cell; the evaluated results generated determine that the biomolecular interactions can be revealed by changes in electrochemical parameters.[14] The sensitivity of this electrochemical system is quantitatively determined, showing that the increase in glucose concentration in the medium promoted an increase in electrochemical potentials.

These analytical sensors were developed based on the ability to change the evaluated electrode/electrolyte interface of a protein with its specific ligand interaction; systems capable

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of determining the charge distribution on protein surfaces, such as lectins, were constructed to define specific antigen-antibody interactions.[14,35,51,52]

Cramoll-glucose cyclic voltammetry

Cyclic voltammetry (VC) is a powerful technique to probe the electrochemical properties of organic molecules;[53] it has been used in the detection of glucose without the presence of lectin,[54] and with the presence of Cramoll.[55]

It was verified that there is no change in the electrical current in a cyclic voltammogram with the gold disk containing only the carbon paste and different concentrations of glucose (10, 15 and 20 mM) in an electrolytic medium of 20 mM potassium phosphate buffer, pH 7.0. After the addition of MOF in this carbon paste, the spectra presented in the cyclic voltammogram (Fig. 3) showed a change in the anodic and cathodic peaks, indicating the possibility of this system being used in the construction of the biosensor after the immobilization of a biomolecule on the surface of this modified electrode.

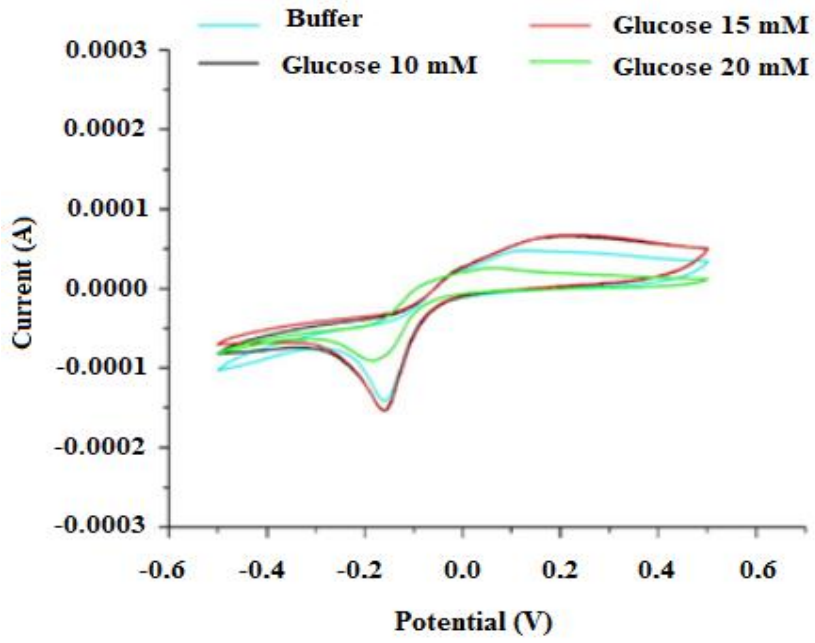

Fig. 3. Gold electrode voltammograms, with carbon paste and MOF in concentrations of 5 mM, 10 mM, 15 mM and 20 mM of glucose and buffer.

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We used the biosensor technique to assess the relationship between the response current and glucose concentration; electrochemical current monitoring assesses lectin-carbohydrate binding using this amperometric method.[55,56] It is observed in the CV graph (Fig. 4) that the standard analysis (in buffered medium) shows a linear increase in current, even after 0.0 V of potential, thus not showing biological interactions.[53] While the samples with glucose showed an exponential increase in the cathodic current, at peak 1, representing the binding site of the monosaccharide, in the potential range from –0.2 to 0.0 V; with the current reaching 0.0003 A, after 0.0 V the current decreases. In this experiment, a concentration of 40 mM glucose was added; the reduction of peak 1 at this carbohydrate concentration reinforces Cramoll saturation indicated by this ligand. We know that the identification of peaks in the voltammogram already provides very valuable information about ongoing processes, since different electrochemical processes generate different characteristic peaks.[57] The study by Souza et al.[14] reaffirms the interaction existing at peak 1 (lectin-carbohydrate), as it was observed that in CV with Cramoll/Nafion, in the absence of glucose, they did not induce redox peaks (maintaining the current around 800 µA), while at glucose concentrations the highest cathodic current response was nearly 1300 µA in the presence of 100-300 mM glucose.

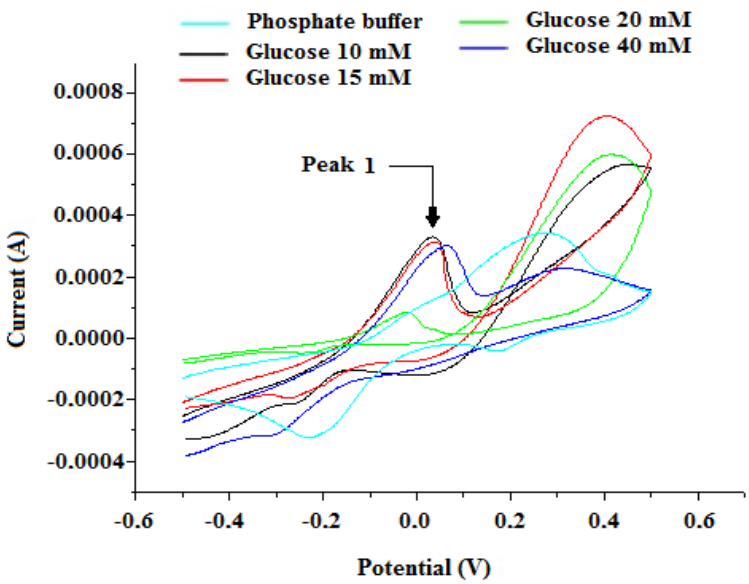

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Fig. 4. Cyclic voltammograms with gold electrode obtained by Cramoll/MOF interaction with different glucose concentrations.

Cramoll/glucose/ rabbit erythrocyte electrochemical potentials

Proteins known as lectins, also called hemagglutinins, have the ability to interact with glycans present on cell surfaces, agglutinate erythrocytes, mediating a series of biological processes.[58] *C. mollis* seeds have hemagglutinating activity[44] and induce the permeability transition of some types of membranes;[9,58] one of the reasons is the interaction of the metallic cation in the membrane structure, forming the lectin-Ca^{2+} interaction.[59] Lectins have been reported as potential tools for recognizing disease-related changes in glycans. Electrochemical immunosensors have already been developed for the specific detection of proteins, biomarkers and pathogens[7,19,60] contributing to the rapid detection of the disease and reducing morbidity and mortality.[61]

Electrochemical assays were also performed with rabbit erythrocytes in order to assess the interactions of Cramoll with glucose, in the buffered medium/glucose/red cell membrane of the erythrocyte, knowing that there are changes in passive electrical parameters (the permissiveness ε and a σ) electrical conductivity of erythrocyte cell membrane, induced by the presence of glucose in the extracellular medium and the presence of proteins, lipids and ions found in the serum.[38,62] Biosensor electrochemical potentials were obtained in the absence of glucose, in buffered medium (Fig. 2B) and in buffered medium/rabbit erythrocytes (Fig. 5A), to be reference standards, looking for differences in the potentiometric curves. According to Souza et al.[45] Cramoll has a redox potential of 94 mV higher than Con A (84 mV) in the same experimental condition; this result indicates a better conformation of Cramoll in the electrochemical system to evaluate the interaction with ligands, an important condition for characterizing the lectin-carbohydrate interaction in biological media.

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Figure 5A and 5B reflects the interaction of Cramoll-rabbit erythrocytes and indicate that Cramoll is not specific for rabbit erythrocytes.[60,63] In the study to measure the changes in the electrochemical potential of the rhizome lectin of *Microgramma vaccinifolia* (MvRL)[35] ion-lectin interactions Ca^{2+} and Mg^{2+} were observed in the absence and presence of human erythrocytes, while when the system was performed in the presence of erythrocytes and absence of Ca^{2+} and Mg^{2+}, the electrochemical potential of MvRL remained constant, revealing that the lectin-Ca^{2+} and lectin-Mg^{2+} potentials did not change in the presence of erythrocytes. In the presence of cations it may indicate that the ion-lectin interaction resulted in conformational change and/or alterations in the lectin surface charge distribution. This study corroborates our results by noting that lectins did not interact with erythrocytes, but did interact with their specific ligands.

Therefore, we suggest that Cramoll 1,4 does not perform the lectin-Ca^{2+} interaction with the Ca^{2+} cation of the rabbit serum membrane, inhibiting its hemagglutinating activity and maintaining its biorecognition activity with glucose (Fig. 5B).

The sensitivity of the electrochemical system to the presence of the Cramoll lectin in the electrolyte medium interacting with carbohydrates from the erythrocyte membrane of rabbits was determined (Fig. 5B). The potential of the potentiometric curves decreased in the interval between 2 to 10 min, and after 10 min, there is an increase in the electrochemical potential, behavior similar to that observed in the phosphate buffer biosensor supplemented with identical concentrations of glucose (Fig. 5B). It has also been observed that the potential decreases when Cramoll is in the electrochemical system; this change is due to the insulating nature of the biomolecule.[51]

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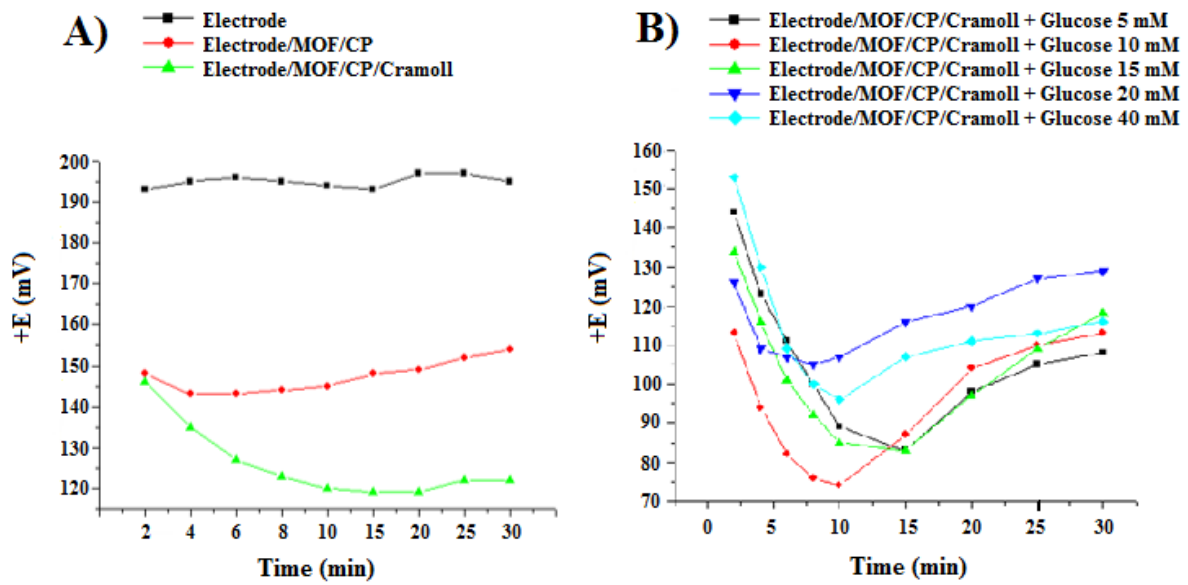

Fig. 5. Electrochemical potentials regarding the interaction in (A) phosphate buffer solution/rabbit erythrocytes and (B) phosphate buffer solution/rabbit erythrocytes at different glucose concentrations.

When lectin-specific carbohydrate is placed in the system with rabbit serum, the hemagglutination capacity decreases.[59,60] The Cramoll/glucose/rabbit erythrocyte electrochemical system demonstrated that even in a complex environment it has selectivity and sensitivity in detecting changes in the electrochemical potentials of Cramoll promoted by the glucose-Cramoll interaction, with the formation of lectin-carbohydrate complexes, small conformational lectin changes occur.[64,65]

Images of the interaction of Cramoll immobilized on the MOF surface with the specific ligand using a scanning electron microscope (SEM)

Along with electrochemical characterization techniques, structural and morphological characterization is of great importance for the analysis and proof of structural and morphological changes on electrode surfaces, especially after the use of polymers, nanomaterials and biomolecules.[51,66]

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SEM is a technique used to investigate the morphology and conformational changes in surfaces, available for the observation and analysis of microstructural characteristics of solid materials, providing very fast information on the morphology and identification of chemical elements in the sample. Another important characteristic of SEM is the three-dimensional appearance of the sample images, a direct result of the large depth of field;[21] in the MOF image (Fig 6A), it was observed that the crystal provides a smooth-surfaced octahedral morphology, as reported in the literature.[23, 67,68]

We used Concanavalin A (Con A) a plant lectin isolated from *Canavalia ensiformis* (*C. ensiformis*) seeds as our control, belonging to the same Fabaceae family as Cramoll and sharing similar characteristic structures and comparable carbohydrate recognition.[69] Cramoll has 82% amino acid sequence identity when compared to Con A (194/236 residues) and 42 substitutions.[70] Con A exists as a tetramer with four carbohydrate binding sites, having its selective mannose and glucose binding sites well-studied.[71-74] In the samples in which the immobilizations of lectins in the MOF were performed: Cramoll (Fig. 6B) and Con A (Fig. 6E), it was observed that different from Fig 6A on the surfaces of the MOF is an adsorbed structure, with the same morphology shown in the SEM[73] which refer to the lectins that have been immobilized in the crystal.

From the qualitative evaluation of the interaction of lectins immobilized on the MOF surface and interacting with glucose: Cramoll 10 (Fig. 6C) and 20 mM (Fig. 6D); Con A (Fig. 6F) and 20 mM (Fig. 6G), which revealed Cramoll/MOF and Con A/MOF interactions with this carbohydrate, the study by Nagae and Yamaguchi,[65] presents a three-dimensional structure of polysaccharide binding proteins, showing how proteins bind to carbohydrates. The control images showed similar results with Cramoll, which according to Roy and Das,[72] SEM reveals morphological changes that occurred due to biological interactions.

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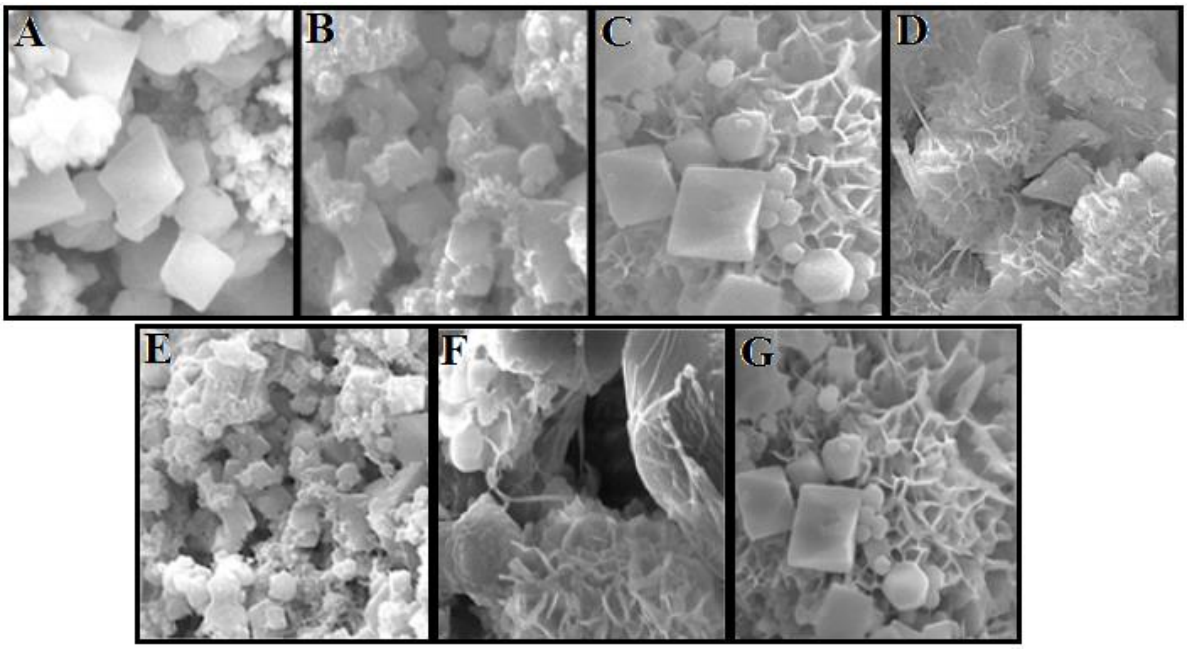

Fig. 6. Scanning electron microscopy (SEM) images. (A) MOF; (B) interaction of Cramoll immobilized on surface MOF, MOF/Cramoll; (C) MOF/Cramoll/10 mM glucose; (D) MOF/Cramoll/20 mM glucose; (E) MOF/Con A; (F) MOF/Con A/10 mM glucose; (G) MOF/Con A/20 mM glucose. Image field: 13.8 µm.

Conclusion

The new electrochemical model with gold/MOF/Cramoll electrode for biological characterization was effective due to its high sensitivity to conformational changes related to specificities of the lectin with free ligand or in the membrane of rabbit erythrocytes in the electrolytic medium, through potentiometric methods (potentiometric electrochemical) and amperometric (cyclic voltammogram). This phenomenon of Cramoll interaction immobilized on the surface of MOF with different concentrations of glucose was also verified through images using a scanning electron microscope (SEM). This MOF/Cramoll biosensor is promising for carbohydrate lectin evaluation at biological interfaces.

Experimental Section

Plant material

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313 Seeds of *Cratylia mollis* Mart. ex Benth. were harvested by Rita Pereira (11/09/1990)
314 in Ibimirim, State of Pernambuco (PE), Northeast of Brazil (8° 32' 26" S/ 37° 41' 25" W). A
315 sample of the collected material is archived as voucher specimen number IPA 54135, at the
316 Herbarium IPA - Dárdano de Andrade-Lima (Instituto Agronômico de Pernambuco - IPA,
317 Recife, PE, Brazil). The plant identification was made by Dr. Rita Pereira, the herbarium
318 curator.
319
320 *Electrochemical Synthesis of the Metal-Organic Framework - MOF of [Cu3(BTC)2.(H2O)3]n*
321 The MOF synthesis of $[Cu_3(BTC)_2.(H_2O)_3]_n$ was performed by the amperometric
322 electrochemical route according to the method developed [68]; the reagents were used without
323 prior purification, as follows, acid 1, 3, 5-benzenetricarboxylic (BTC) with 98% purity, N,N-
324 dimethylformamide (DMF) 99.8%, sodium nitrate 99%, two copper plates 99% and water
325 with high purity (Millipore®). Copper plates were used as auxiliary and sacrificial work
326 (consumed by oxidation) electrodes. These electrodes underwent physical (water sandpaper)
327 and chemical pre-treatment, emerging an area of 5 cm^2 in nitric acid 20% by volume, for 2
328 min. The system consisted of a single-compartment Pyrex glass electrochemical cell. The
329 electrochemical route was amperometric, keeping the potential at 12V using the DC POWER
330 SUPPLY model PS-1502 DD source. The solution used was a mixture of 1,3,5-
331 benzenetricarboxylic acid (0.048 mol L^{-1}), solubilized in a mixture of DMF/H_2O (1/1)
332 solvents. After 17 min of reaction a MOF blue solid was obtained in 73% yield. This solid
333 was filtered, washed with the same mixture of solvents and taken to dryness in an oven at a
334 temperature of 120 ºC for 30 min.
335
336 *Immobilization of Cratylia mollis (Cramoll) seed lectin on MOF/platinum electrode and*
337 *MOF/gold electrode surface*

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For immobilization, 0.0060 g of MOF was used and 20 µl of Cramoll were added to the platinum electrode. In the construction of the gold electrode (microcavity corresponding to 0.03 mm) 0.0020 g of MOF and 10 µl of lectin were used. To fix the MOF/Cramoll on the platinum and gold electrodes, a paste composed of 0.0500 g of powdered carbon and 30 µL of mineral oil was used (Fig. 7), thus the systems corresponded to the characteristic patterns of a biosensor.[51]

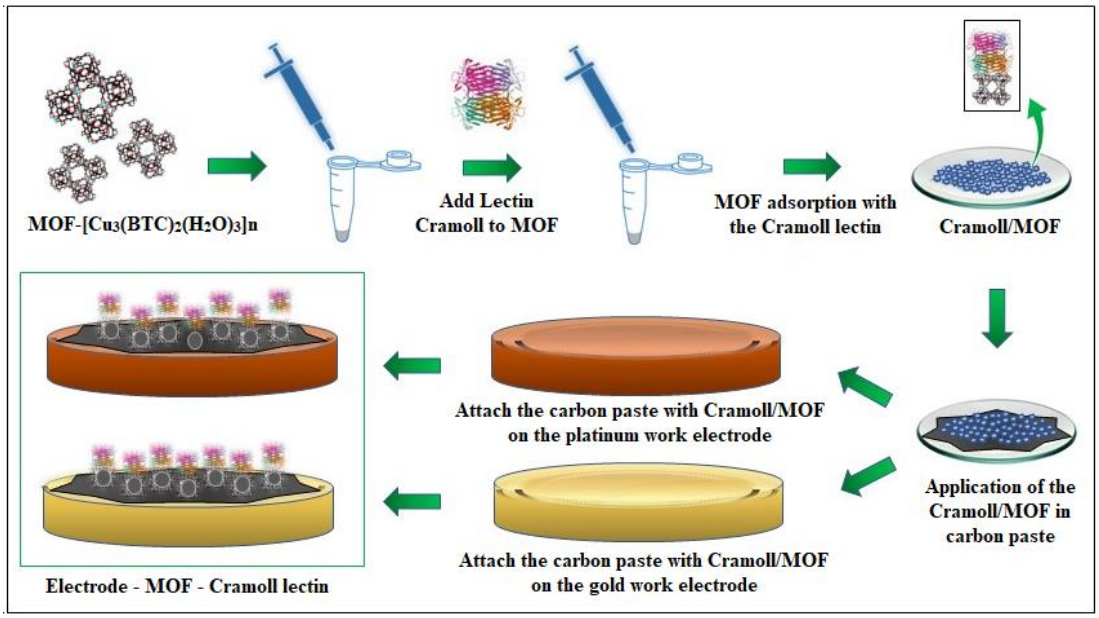

Fig. 7. Schematic MOF immobilization on a working electrode.

Electrochemical potential

To obtain the electrochemical potential due to the interaction of Cramoll in an electrolytic medium composed of 200 mM potassium phosphate buffer and pH 7 with glucose concentrations of 5, 10, 15, 20 and 40 mM, an electrochemical cell containing a silver reference electrode/ silver chloride (Ag/AgCl) and working electrode (platinum and gold electrodes) were used, with the Cramoll lectin immobilized on the crystalline polymer MOF-$[Cu_3(BTC)_2(H_2O)_3]n$.

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Cyclic Voltammetry

Measurements of cyclic voltammetry were carried out using a Potentiostat/Galvonostat/Metrohm, PGSTAT 302 N and software GPES 4.9, with an electrochemical window of initial potential from -0.5 V to 0.5 V with scanning potential of 50 mV/s. Voltammetry was previously performed in glucose and fructose solutions at different concentrations (20 ml, 40 ml, 60 ml, 80 ml and 100 ml) diluted in phosphate buffer (pH= 7) using the platinum electrode as the working electrode. Voltamograms were also obtained with 5, 10, 15, 20 and 40 mM glucose, in buffered medium (pH = 7), employing the gold electrode as the working electrode.

Obtaining electrochemical potentials with the addition of erythrocytes

To evaluate the specific interactions of Cramoll, we performed the reference electrochemical potentials, where the first standard was constituted by an electrolytic medium (200 mM phosphate buffer) and the second standard was related to the buffered medium with rabbit erythrocytes. For the characterization of Cramoll-glucose, glucose concentrations of 5, 10, 15, 20 and 40 mM and rabbit erythrocytes (2.5%, suspension, v/v) in 200 mM phosphate buffer electrolytic medium were used. All electrochemical potentials were performed in a biosensor composed by an electrochemical cell containing the gold electrode and the reference electrode (Ag/AgCl), both connected to the potentiometer with a cable adapted for potentiometric readings.

Obtaining images with a scanning electron microscope (SEM)

The images of MOF, MOF immobilized on Cramoll and the characterization of the MOF/Cramoll interaction with different glucose concentrations (5, 10, 15 and 20 mM) were performed; for a standard the MOF/Con A. All image acquisitions were made through the

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scanning electron microscope (SEM), equipment (TESCAN, Model: VEGA3) available at CENAPESQ/UFRPE.

Author Contributions Statement

M.H. Carvalho, S.R. Souza and L.C.B.B. Coelho designed the studied protocol; M.H. Carvalho, H.D.A. Araújo, R.P. Silva, M.T.S. Correia, K.C.S. Freitas, S.R. Souza and L.C.B.B. Coelho carried out the assays and were involved in analysis and interpretation of all data; M.H. Carvalho, H.D.A. Araújo, S.R. Souza and L.C.B.B. Coelho contributed to drafting the manuscript and/or critically revising the paper and intellectual content. All authors read and approved the final manuscript.

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Conflict of interests

The authors have no conflict of interest.

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