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Cite as: AIP Advances **6**, 111302 (2016); https://doi.org/10.1063/1.4966559
Submitted: 31 July 2016 • Accepted: 22 September 2016 • Published Online: 21 November 2016

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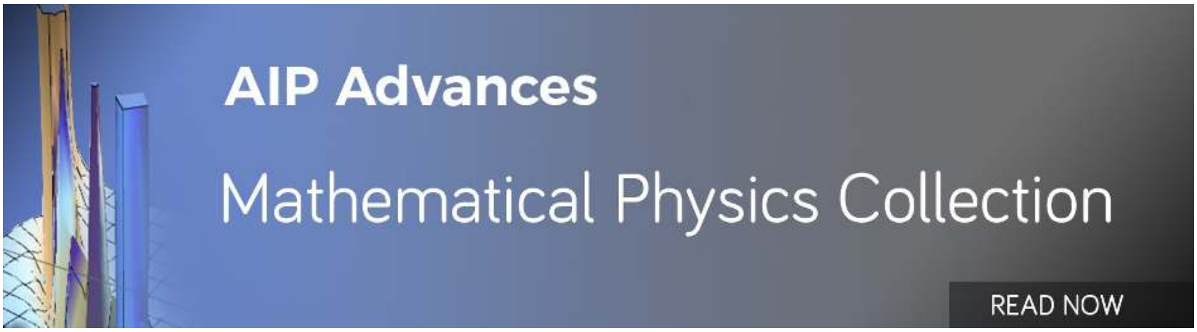

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Polysaccarides-based gels and solid-state electronic devices with memresistive properties: Synergy between polyaniline electrochemistry and biology

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(Received 31 July 2016; accepted 22 September 2016; published online 8 November 2016)

A new architecture of organic memristive device is proposed with a double-layered polyelectrolyte, one of which is a biological system that alone drives the memristive behavior. In the device the Physarum polycephalum was used as living organism, the polyaniline as conducting polymer for the source-drain channel. The key choice for the device functioning was the interposition of a biocompatible solid layer between polyaniline and living organism, that must result both electrochemically inert and able to preserve a good electrical conductivity of the polyaniline, notwithstanding the alkaline pH environment required for the surviving of living being, by avoiding strong acids. Pectin with a high degree of methylation and chitosan were tested as interlayer, but only the first one satisfied the essential requirements. It was demonstrated that only when the living organism was integrated in the device, the current-voltage characteristics showed the hysteretic rectifying trends typical of the memristive devices, which however disappeared if the Physarum polycephalum switched to its sclerotic state. The mould resulted to survive a series of at least three cycles of voltage-current measurements carried out in sequence. *© 2016 Author(s). All article content, except where otherwise noted, is licensed under a Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).* [http://dx.doi.org/10.1063/1.4966559]

I. INTRODUCTION

The integration of electronic devices and biological objects or even living organisms has recently attracted considerable interest of scientists.[1–5]

The emulation of unique abilities of living beings by electronic devices is one of the main goals of bioelectronics. It was shown that polyaniline-based memristive networks[6–9] were able to reproduce a part of the pond snail nervous system (Hebbian learning).[10]

Research frontiers are also oriented towards hybrid systems, where biological systems are key components of electronic devices.[11–13] For example, the adaptive 'learning' behavior of slime mould Physarum polycephalum (PPM) was described in terms of memristor model;[14,15] the mould also resulted to be able to perform complex tasks of the information processing.[16–18] Gale et al.[19] observed occasionally that PPM protoplasmic tubes showed hysteretic current-voltage characteristics, consistent with those of the memristive systems.

Although polyaniline (PANI) based devices have considerably progressed in recent years, the use of polyaniline in biosensors or biological integrated systems has serious limitations because the most of enzymatic and cellular activities are pH sensitive. The polyaniline becomes a very poor conductor at pH values higher than 5, required for biological objects.

In this study a strategy to match polyaniline and biological system in the same device is proposed by introducing an intermediate layer between polyaniline and biological object. The interface layer

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was a “solid buffer” able to retain the PANI conductivity. Moreover, it should be electrochemically “inert”, thus it does not interfere with the ion exchange between biological component and PANI: asymmetric, hysteretic current-voltage characteristics are required to be exclusively due to interaction between biological component and PANI. To preserve biocompatibility, strong acids were avoided and polysaccharides-based materials preferred. The PPM was used as a biologic component; two biopolymers were investigated, chitosan and pectin.

Chitosan, a copolymer of glucoseamine and N-acetyl glucosamine, is a natural biopolymer obtained from chitin, with different acetylation degree (DA). Chitosan (CS) is soluble in dilute acidic solutions at pH lower than 6.0, giving gels at high concentrations.[20]

Pectin has a 1,4-linked α-D-galacturonic acid polysaccharide backbone. The formation mechanism of pectin-gel depends on esterification degree (DE): based on coordination bonding with bivalent ions (e.g. Ca^{2+}, Mg^{2+}, etc.) in low methoxyl- (LM) pectins, and on hydrogen bonding and hydrophobic interaction in high metoxyl- (HM) pectins.[21]

The properties of chitosan and pectin-gels were extensively studied.[22,23] These biopolymers as hydrogels, films, fibers, sponges were employed in numerous applications requiring good biocompatibility: in food industry,[24,25] in drug delivery,[26] for bone tissue engineering,[27] etc.

II. MATERIALS AND METHODS

Chitosan (poly(D-glucosamine) medium molecular weight (Mw∼100KDa), from Pandalus Borealis, 77% deacetylation), pectin (poly-D-galacturonic acid methyl ester, Mw 30000-100000 Da, from apple etherification degree 70-75%), polyethylene oxide (PEO) (Mw∼8000000 Da), emeraldine base polyaniline (Mw∼100000 Da) were purchase from Sigma-Aldrich. N-methyl pyrrolidone (Sigma-Aldrich, anhydrous, 99.5%), toluene (Sigma-Aldrich, ACS reagent, ≥99.5%), acetic acid (Fluka, ACS reagent, 0≥99.7%), hydrochloric acid (Fluka, ACS reagent, 37%) were used without further purification.

Water (for film deposition, solution preparation, washing) was purified by Milli-Q system (resistivity: 18.2 MΩ cm).

Strain of slime mold Physarum polycephalum (Order Physarales, class Myxomecetes, subclass Myxogastromycetidae) was obtained as a sclerotium from Bristol University (UK). The mold was grown in humidity-controlled chamber with 1,5% Agar non nutrient gel.

Chitosan-gel (50 mg/mL) and high methoxyl pectin- (PECHM) gel (50 mg/mL) were prepared in 2% acetic acid. They were used as solid polyelectrolytes.

The electronic memristive devices were prepared following the procedure proposed by Berzina *et al*.[8] Briefly, the deposition of PANI layers was carried out with a KSV 5000 LB trough, using a modified Langmuir-Schaefer (LS) technique.[28] Sixty molecular layers with a total thickness of about 100 nm were deposited onto glass substrates with chromium electrodes. The resulting film was doped in 1M HCl for 2 min to transfer PANI into its conductive emeraldine salt state. This layer formed a conducting channel between source (S) and drain (D) electrodes. A stripe of gel based on solid polyelectrolyte of about 1-2 mm wide was deposited in the central part of the PANI channel by solution casting. Ag wire (50 µ in diameter) was attached to the polyelectrolyte stripe and was called gate electrode (G). In standard procedure[8] the assembled structure was additionally doped with HCl (37%) vapor. Source (S) and gate (G) electrodes were connected to the ground potential level, while the variable voltage was applied to the drain (D) electrode (figure 1).

The electrical measurements were performed using a Keithley 236 source measure unit and a Keithley 6514 system electrometer. The 236 unit was used to apply a potential difference between the source (S) and the drain (D) electrodes of the device, keeping the source at the ground level, and to measure the total current ID passing through the device. The 6514 unit was used to measure the current passing from the gate to the drain electrode IG, i.e. the ionic current. The subtraction of this value from the one measured by the 236 unit gives the value of electronic current passing through the PANI layer. The voltage–current characteristics were measured between −1.2 V and +1.2 V, in a closed loop with the sequence 0 V, +1.2 V, -1.2 V and coming back to 0 V, with steps of 0.1 V. Readout of the current value at each voltage step was performed 60 s after the

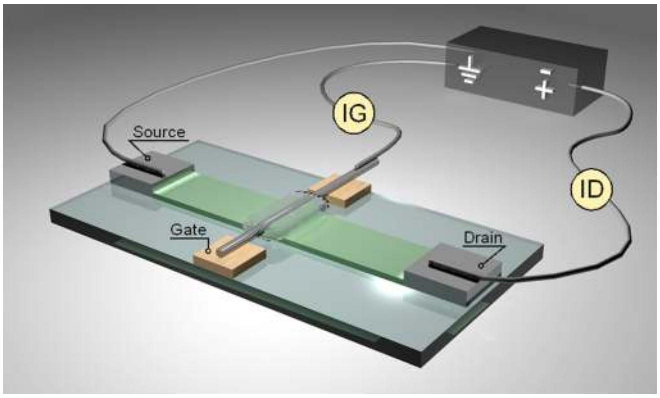

FIG. 1. Schematic representation of organic memristive device and circuit for measuring of its voltage-current characteristics.

setting of the voltage (delay time), in order to leave time for the transient processes in the device to equilibrate.[9]

III. RESULTS AND DISCUSSION

In the first step of the study, we assembled devices following the standard procedure[8] but using chitosan or pectin-gels as solid polyelectrolytes. Then, we achieved the best conditions to preserve high conductivity of polyaniline at neutral pH and to assure linear electrical characteristics of the device. Finally, we realized a multi-layered structure by inserting a biological element and tested the performances of this bio-integrated electrochemical device.

A. Combination between polysaccarides-based gel and polyaniline layer

At first, the device was prepared using chitosan-gel (in 2% acetic acid) as a solid electrolyte, HCl (37%) was added, as in previous studies.[8] The current-voltage characteristics exhibited clear memristive features, hysteresis and rectification (figure 2 top), without significant variations even if $LiClO_4$ was added.

The current-voltage characteristics of chitosan-gel stripes in contact with different electrodes (silver, chromium, platinum) were studied also without PANI, to understand if an eventual redox reaction at the biopolymer-electrode interface takes place inducing asymmetric I-V characteristics, alternatively to the redox reaction at the PANI-biopolymer interface. Figure 3 (bottom) shows the I-V characteristic of a chitosan-gel layer doped with HCl and $LiClO_4$.

Linear current-voltage characteristics were observed; this excludes that reactions at electrodes or polyelectrolyte degradation affect the memristive behavior (figure 1). Therefore, chitosan resulted suitable as electrochemically “inert” material. No significant differences in the electrical data were observed, in comparison, for PEO (figure 3 top) (PEO is used in Refs. 8 and 9).

The next step of the study concerns the substitution of HCl (37%) as dopant (used in Refs. 8 and 9) because HCl is not suitable for the realization of bio-integrated devices: mild conditions are needed and high concentration of Cl^- ions (40g/L in standard devices[8,9]) must be avoided. Then, to eliminate strong inorganic acids and any ionic species in the device, chitosan- and pectin-based gels were also prepared in aqueous solutions of diluted inorganic acids without addition of salts such as $LiClO_4$.[29]

A different behavior was observed for chitosan and pectin-gels in contact with PANI layer. By using chitosan-gel without strong acid, PANI layer switched into the emeraldine base insulating form (≥200 MΩ), even if the acetic acid content was increased up to 30%. In these conditions it was impossible to acquire current-voltage characteristics. On the contrary, when the pectin-based gel with acetic acid 2% w/w was combined with PANI, it did not induce the de-doping of the polymer, in spite of being a water-based gel, but rather decreased its resistance (≤50 kΩ). Cyclic

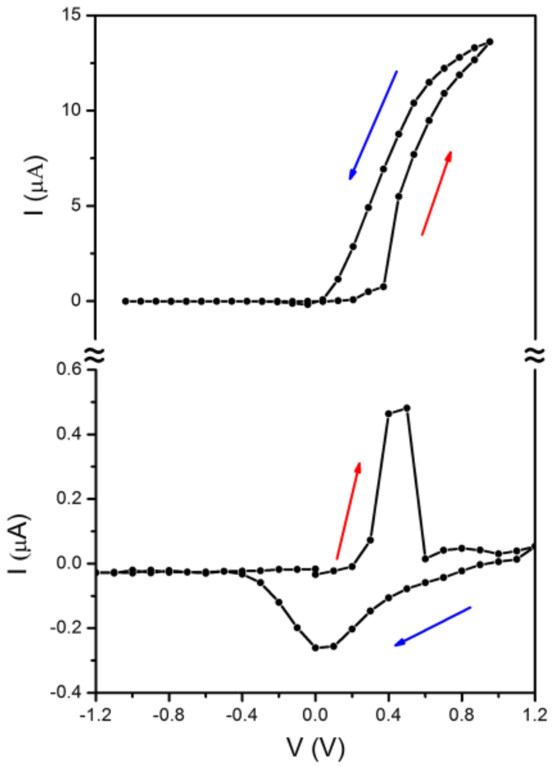

FIG. 2. Cyclic voltage-current characteristics of organic memristive device with solid polyelectrolyte stripe made of chitosan-gel with addition of HCl for electronic (top) and ionic (bottom) currents. Arrows indicate the direction of the voltage variation.

voltage-current characteristics were measured with and without adding strong acids in gel composition. In both cases, current-voltage characteristics were detectable, with current of the order of μA, even if with different trends: typical memristive characteristics for pectin-gel with HCl 0,1M (figure 4 top) and linear characteristic for pectin-gel without HCl, (figure 4 bottom). To explain different behaviors observed for chitosan- and pectin-gel, we propose the following observations. The acetic acid concentration (2% w/w) used for chitosan solubilization and gelification should give a pH value (2.4) capable to preserve, in theory, PANI in conductive state. In our experimental conditions, chitosan (pKa 6.3) formed quaternary nitrogen salts becoming a water-soluble cationic polyelectrolyte,[30] whose counter ion, being involved in the charge balance, is the anion acetate.

The protonation of amino groups of chitosan shifted the acetic acid dissociation equilibrium towards the anion acetate formation. The H^+ concentration, mainly involved in chitosan protonation, was not sufficient to preserve PANI in the conductive state owing to basic hydrolysis of the acetate ion. Even high acetic acid concentration (up to 30% w/w) was not able to preserve the PANI conductivity. Concentrations of acetic acid higher than 30% w/w were not considered, because they induced over-oxidation of PANI and chitosan depolymerization, as the gel softening showed, according to literature.[31,32] Thus, we can conclude that chitosan-gel, although it has a good biocompatibility, is not able to preserve the PANI conductivity without adding of strong acid.

The pectin-based gel, instead, was prepared using pectin with high degree of methylation (>74.0% PEC_{HM}), in which hydrogen bonds and hydrophobic interactions are the main factors that induce gelification, rather than pH value or ionic strength.[24,25,33] In any case, as the pH is lowered, ionisation of few carboxylate groups in the PEC_{HM} structure is suppressed, this favors the gelification process.[23] In PEC_{HM}, the acetic acid is slightly involved in protonation process of carboxylate groups and does not participate significantly in gelification of the polysaccharide. Therefore, the concentration of available H^+ ions was sufficient to preserve the PANI conductive state.

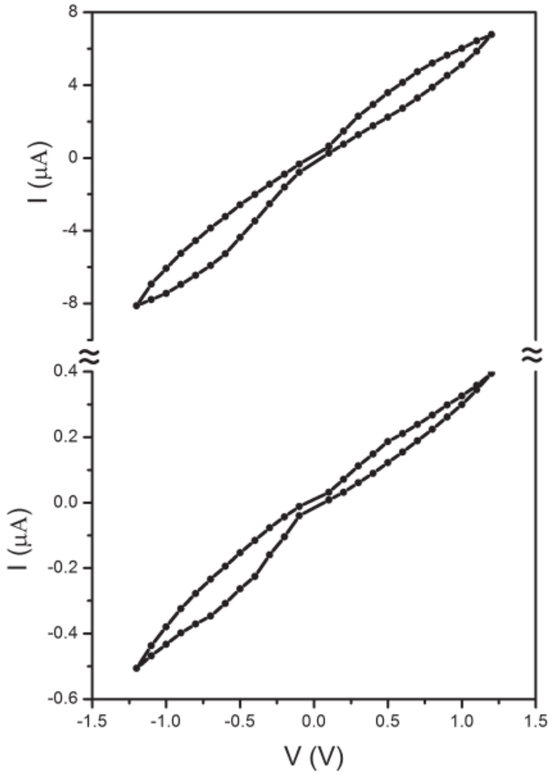

FIG. 3. Cyclic voltage-current characteristics of solid polyelectrolyte stripe, made of chitosan-gel (bottom) compared to PEO-gel (top), with addition of HCl and $LiClO_4$. Direct current mode was used with applied voltage between +1.2 V and -1.2 V for several delay times in the range from 3s to 360 s.

In addition, the different behavior of pectin- and chitosan-gels in contact with PANI layers could be explained on the basis of water uptake by their three-dimensional polymeric networks, which can be a possible reason of PANI de-doping.

B. Bio-integrated devices

The ability of the pectin-gel to act as buffer layer in the device was tested by introducing the PPM as a model living organism. A multi-layer structure was assembled, depositing the living organism onto a dried pectin layer in the contact zone with PANI channel.

Cyclic I-V characteristics were carried out in darkness at relative humidity of 80-90%, respecting the required conditions for the mold life. The electrical circuit was the same as for the standard organic memristive devices.

The interaction between electronic device based on conductive polyaniline and PPM can be understood by considering the ionic species of the mould.

It is known that changes in the PPM membrane potential take place in motor cells, which control the slow movements of the plasmodia related to strands of endoplasm squeezed by alternating contractions of Ca^{2+}sensitive actomyosin fibres.[34–36] If the mould is induced to move by a stimulus into the surrounding environment, like the presence of polysaccharides beneath it, the motor cell permeability to K^+ and Cl^- increases, while the Ca^{2+} ions alter the membrane properties, triggering large fluxes of K^+ and Cl^-. Moreover, the penetration of water inside or outside the motor cell changes the motor cell turgor and promotes the ionic exchanges.

The image of PPM after three successive cycles of I-V measurements is shown in figure 5. The brilliant yellow color of Plasmodium assures that the organism remained alive. In fact, the mould resulted to survive a series of at least three cycles of voltage-current measurements carried out in sequence. Anyway, we would like to note that the factors limiting the device lifetime concern mostly the experimental conditions necessary for the survival of the living organism, such as humidity and

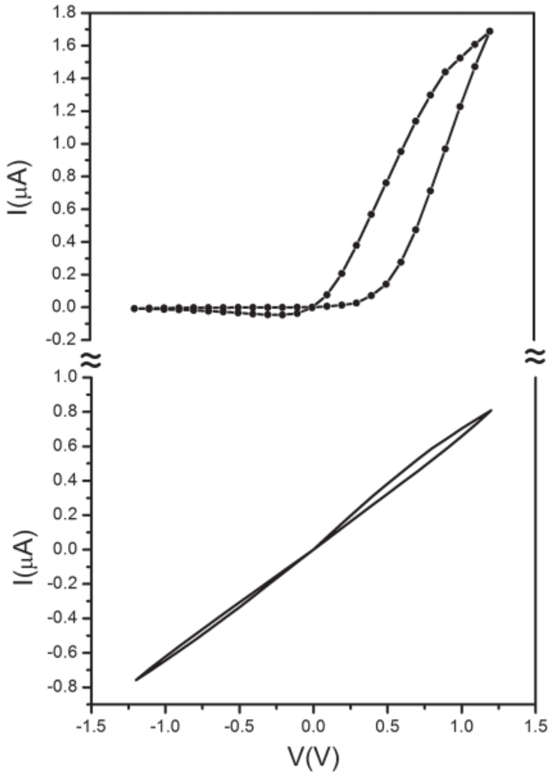

FIG. 4. Cyclic voltage-current characteristic (electronic current) of organic memristive device with solid polyelectrolyte stripe made of pectin-gel with addition of HCl (top) and without addition of HCl (bottom).

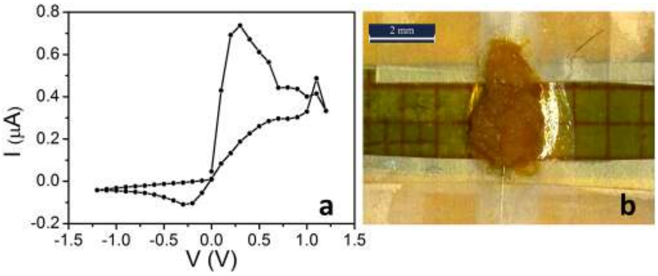

FIG. 5. (a) Cyclic voltage-current characteristic of electronic current in a hybrid organic memristive device with PPM as electrolyte over interfacing layer of pectin-gel; (b) Optical microscope photograph of PPM after three consecutive cycles of V-I measurements.

feeding, rather than current compliance or voltage value. When the mould switches in its sclerotic phase, after making the experimental conditions unfavorable for the life of the mold, it darkens and the current-voltage characteristic loses the memristive features, becoming simply linear, although the PANI layer retains its conductivity. Figure 5a also shows that the PPM plasmodium is able to grow and develop on the layer of pectin, thus demonstrating the biocompatibility of the interface layer.

IV. CONCLUSIONS

A new organic memristive device, interfaced with a biological object through a biocompatible solid polyelectrolyte, was developed and optimized. The polyaniline was the conductive polymer for the source-drain channel. The difficulty to employ polyaniline in bio-integrated electrochemical devices was overcome through the introduction of a biocompatible solid layer, which must result electrochemically inert and able to preserve the electrical conductivity of the polyaniline notwithstanding the alkaline pH, is necessary for living being.

The electrical properties of pectin- and chitosan-gels in contact with polyaniline were investigated. Only pectin, used at high degree of methylation, without strong acids and doped only by acetic acid (2% max), resulted suitable for the purpose. An explanation for the different behaviors of the two polyelectrolytes was suggested. Finally, a Physarum Policephalum was integrated in the device: a hysteretic-rectifying trend, typical of memristive devices, appeared in current-voltage characteristic, what is happening because of the ion exchange through the living mould.

In conclusion, for the first time, the interfacing and implementation of a simple living organism with an electronic device based on polyaniline was successfully achieved, fabricating a hybrid element that could be considered a step towards novel computational techniques. The "double-layer gel method" developed in this work can be extended to other materials and opens the possibility to assemble novel organic bio-integrated devices.

ACKNOWLEDGMENTS

This work has been financially supported by: European project FP7-ICT-2011-8 "PhyChip-Physarum Chip: Growing Computers from Slime Mould" Grant Agreement n.316366. The Provincia Autonoma di Trento, call "Grandi progetti 2012", project "Madelena."

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