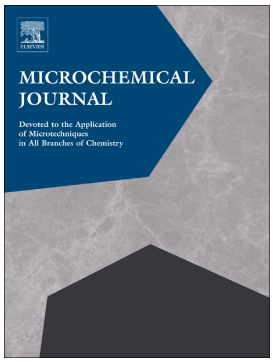

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Evaluation of New Cholinium-Amino Acids based Room Temperature Ionic Liquids (RTILs) as Immobilization Matrix for Electrochemical Biosensor Development: Proof-of-Concept with *Trametes Versicolor* Laccase

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Abstract

In this work, we present new cholinium-amino acids room temperature ionic liquids (ChAARTILs) that can be used as an efficient immobilization matrix for electrochemical biosensor development. The ideal immobilization strategy should be able to ensure the highest enzyme loading and a tight enzymatic immobilization, preserving its native structure and biological activity. In this regard, ChAARTILs present different side chains on the amino acids giving rise to van der Waals, π-π stacking and hydrogen bonding interactions. All these interactions can affect the nanomaterial organization onto the electrode surface. To this aim, we have evaluated the main electrochemical parameters, namely electroactive area (A_{EA}) and the heterogeneous electron transfer rate constant (k^0), showing how both cations and anions of room temperature ionic liquids (RTILs) can independently affect multi-walled carbon nanotubes (MWCNTs) organization. In particular, [Ch][Phe] showed the best performance in terms of A_{EA} (3.432 cm^2) and k^0 ($4.71 \cdot 10^{-3}$ cm s^{-1}) with a homogeneous distribution of MWCNTs bundles onto the electrodes and a faster electron transfer rate. Finally, the modified electrode (MWCNTs-[Ch][Phe]) has been tested with a model enzyme, namely *Trametes Versicolor* laccase (Tvl), in order to evaluate the possibility to use ChAARTILs as immobilization matrix, preventing enzymatic denaturation phenomena which would affect the biosensor performance in terms of sensitivity, linear range, and stability.

Keywords: cholinium-amino acids ionic room temperature liquids (ChAARTILs), multiwalled carbon nanotubes (MWCNTs), *Trametes versicolor* laccase (*TvL*), immobilization strategy, polyphenol index biosensor.

1. Introduction

The main aspect to develop efficient biosensors with appropriate performances is an effective enzyme immobilization (e.g. good operational and storage stability, high sensitivity, high selectivity, high reproducibility and short response time) [1-3]. The ideal immobilization strategy should be able to preserve the enzyme native structure and its biological activity meanwhile the highest enzyme loading and a tight enzyme immobilization are ensured [3, 4]. In the last decades, several immobilization procedures such as physical entrapment, cross-linking, adsorption, affinity and covalent coupling have been investigated in order to develop electrochemical biosensors [5, 6].

In particular, room temperature ionic liquids (RTILs) are compounds based on organic cations and different kinds of anions being in the liquid state at room temperature [7]. During the last century, RTILs have been deeply investigated because of their unique chemical and physical properties, such as high chemical and thermal stabilities, wide electrochemical windows, negligible vapor pressure, high ionic conductivity, low toxicity, and ability to dissolve a wide range of organic and inorganic compounds [8]. However, RTILs have been considered as a suitable media for bioelectrocatalytical processes as reported in the literature [9-12]. Several studies have shown a strong hydrogen bonding ability that is useful for dissolving enzymes preventing protein unfolding or denaturation and creating a favourable environment for the enzyme immobilization [13]. RTILs are able to retain, even enhance, the enzymatic activity [14]. For example, Hinckley et al. reported the catalytic properties of different oxidative enzymes (such as laccase C *Trametes sp.* and horseradish and soybean peroxidases) in a number of ionic liquid-containing systems [15].

The first synthesis of 20 ionic liquids obtained from natural amino acids (AARTILs) and the 1-ethyl-3-methylimidazolium (Emim) cation was reported by Fukumoto's group [16]. Our co-workers recently reported on a new synthesis method for cholinium-amino acids RTILs based on potentiometric titration. This method has several advantages such as shorter preparation time, stoichiometry within ±1%, very high yields (close to 100%), high reproducibility, and no use of organic solvents, thus being more environmentally friendly [17].

The presence of aromatic groups on same amino acids side chains could give rise to several kinds of noncovalent interactions, such as hydrogen bond, π-π aromatic stacking and van der Waals forces

[18-20]. All these interactions are useful for the enzyme immobilization, preserving its activity and structure [21, 22].

Nanomaterials are widely used in biosensors field to enhance the device sensitivity [23-26]. Among them, carbon nanotubes (CNTs) show remarkable electrical, chemical, mechanical and structural properties [27]. As reported by Davis et al. [28], CNTs can display metallic, semiconducting and superconducting electron transport, possess a hollow core suitable for storing guest molecules and have the largest elastic modulus of any known material. CNTs can be divided into single-walled carbon-nanotubes (SWCNTs) and multi-walled carbon nanotubes (MWCNTs) [29]. SWCNTs possess a cylindrical nanostructure formed by rolling up a single graphite sheet into a tube, while MWCNTs comprise of several layers of graphene cylinders, that are concentrically nested like rings of a tree trunk [30-32]. For these reasons, in the last years, several types of research employing CNTs as biosensors components to enhance enzyme loading and electroanalytical performance have been conducted [33, 34].

This paper is aiming at the evaluation of new cholinium-amino acids based RTILs as a novel immobilization matrix for the development of enzymaticbased biosensors, employing *Trametes versicolor* laccase as a proof-of-concept enzyme, because of its ability to undergo both in direct and mediated electron transfer.

The GC working electrodes will be modified by using COOH activated MWCNTs and different cholinium-amino acids based RTILs (ChRTAAILs). The considered amino acids forming the ionic liquids are: Glycine (Gly), Alanine (Ala), Serine (Ser), Cysteine (Cys) and Phenylalanine (Phe); also BmimPF_6 (1-Butyl-3-methylimidazolium hexafluorophosphate) has been tested for comparison. The aforementioned ChAAILs will be investigated in order to find out the best immobilization support (based on the electroactive area A_{EA}, heterogeneous electron transfer rate constant k^0 and the roughness factor ρ) for enzymatic biosensors development.

2. Experimental

2.1 Materials

The five choline-amino acids ionic liquids Choline-Glycine [Ch][Gly], Choline-Alanine [Ch][Ala], Choline-Serine [Ch][Ser], Choline-Cysteine [Ch][Cys] and Choline-Phenylalanine [Ch][Phe] (see structures in figure 1A SM) were synthesised as reported by Masci et al. [17]. Choline hydroxide and all AAs were purchased respectively from Fluka and Sigma Aldrich. 1-Butyl-3-methylimidazolium bis(trifluoromethanesulfonyl)imide (Bmim NTF_2), whose structure is reported in figure 1B SM, was obtained from IOLITEC Ionic Liquids Technologies GmbH Germany.

Multiwalled carbon nanotubes, obtained by chemical vapor deposition method (MWCNT, O.D. × I.D. × L: 10 nm × 4.5 nm × 4 μm), were supplied from Sigma Aldrich (Sigma Aldrich, SouthWest NanoTechnologies, Inc., 2501 Technology Pl, Norman, OK, USA). Potassium hexacyanoferrate (III) ($K_3[Fe(CN)_6]$), acetic acid (CH_3COOH), sodium dihydrogen phosphate (NaH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), sodium acetate (CH_3COONa), glutaraldehyde solution (GA, Grade I, 25% in H_2O) were obtained from Sigma-Aldrich (Sigma Chemical Company, St. Louis, MO, USA). Fungal Laccase from *Trametes versicolor (Tv*L, EC 1.10.3.2, activity: 30.6 U mg^{-1}) was supplied from Sigma-Aldrich and stored at -18 °C.

All solutions have been prepared by means of high purity deionized water (Resistance = 18.2 MΩ cm at 25 °C; TOC < 10 μg L^{-1}, Millipore, Molsheim, France).

2.2 Equipment

Scanning electron microscopy (SEM) measurements were performed with High-Resolution Field Emission Scanning Electron Microscopy (HR FESEM, Zeiss Auriga Microscopy, Jena, Germany) equipped with Microanalysis EDS ≤ 123 Mn-Kα eV (Bruker Italia S.r.l., Milano, Italy). All samples were prepared according to the modification protocol, as reported in section 2.3, using glassy carbon plates (25 × 25 × 1 mm, ALS Co. Ltd., Tokyo, Japan) instead of GC electrodes.

Cyclic voltammograms (CVs) were recorded by using a μ-Autolab Type III potentiostat (equipped with GPES 4.9, Autolab, Utrecht, The Netherlands). CVs were performed in a three-electrode electrochemical cell containing a standard calomel electrode (SCE, Amel, Milano, Italy), a graphite rod counter electrode and a modified glassy carbon (GC) electrode as the working electrode. The modified GC electrode, an SCE reference electrode, and a platinum wire counter electrode were fitted into a wall-jet cell. The electrochemical system was equipped with a flow system consisting of a peristaltic pump (Gilson, Villier-le-Bel, France) and a six-port valve electrical injector (Cheminert® Injection Valves for FIA Model C22 Sample Injector - 1/4-28 fitting, Seattle, WA, USA) with a 50 μL injection loop was used for the injection of analytes into the carrier flow (constant flow rate of 0.5 mL min^{-1}) [35]. All measurements were performed in 50 mM acetate buffer pH 5 except for the electrochemical characterization of MWCNTs-COOH/ ChAARTILs that was performed in 50 mM PBS buffer pH 7.4.

2.3 Preparation of the MWCNTs-COOH/ChAARTILs modified electrodes and biosensor assembling

GC electrodes (Metrohm, Switzerland, GCE, Cat. 6.1204.300, ∅=3 mm) were polished with alumina slurries (Al_2O_3, particle size of 0.3 and 0.05 μm) on cloth pads wet with Milli-Q water (SIEM,

Bologna, Italy), thoroughly rinsed with Milli-Q water and further sonicated for 5 min between each polishing step.

MWCNTs were purified and activated by stirring in 2 M nitric acid solution for 20 h. The solid product was collected on a filter paper and washed several times with deionized water up to neutrality. The so activated MWCNT-COOHs were dried in an oven at ~80 °C for 24 hours. This procedure ensures the complete removal of transition metal ions, used as catalysts in the production of carbon precursors. The oxidative process produces carboxyl groups on the MWCNT surface [36]. 1 mg of activated MWCNTs-COOH was re-suspended in 100 mM aqueous solution of each ChAARTILs. Afterward, 4 µL of MWCNTs-COOH/ChAARTILs suspension were drop-cast onto the GC electrode and let it dry overnight at room temperature [37]. The best-modified electrode was selected on the basis of the electroactive (A_{EA}), heterogeneous electron transfer rate constant (k^0, cm s^{-1}) and roughness factor (ρ), calculated from CV measurements carried out in 1 mM $Fe(CN)_6^{3-/4-}$ (50 mM PBS buffer pH 7.4). Finally, the enzyme immobilization was performed by mixing *Trametes versicolor* laccase with MWCNTs-COOH/ ChAARTILs suspension and further cross-linked with glutaraldehyde (2,5 % v/v in distilled water) [38]. The so modified electrodes were let to dry overnight at room temperature and stored in 50 mM acetate buffer pH 5 at 4°C.

All the electrochemical measurements were performed by using three individual assemblies of working electrodes and three replicate measurements for each.

2.4 Polyphenol content by Folin–Ciocalteau method

For comparison, wine samples were also analyzed by the spectrophotometric method involving the use of the Folin–Ciocalteau (FC) reagent using a T60U Spectrometer PG Instruments Ltd (Wibtoft Leicestershire, United Kingdom). Briefly, 100 µL of white wine sample, or 20 µL in the case of red wines, were added to 2000 µL of chromogen solution; the same procedure (using the same volume, respectively) was also adopted for the blank (distilled water) and standard (0.6 g L^{-1} gallic acid for white wines or 3 g L^{-1} gallic acid for red wines). The obtained solutions were mixed and incubated for about 1 min at room temperature; 1000 µL of alkaline buffer were added to every solution which were then mixed and incubated for 30 min at room temperature. The absorbance of the blank sample and standard solutions were read at 760 nm against distilled water. This method is specific for the –OH groups of polyphenolic compounds, the obtained result is referred to the polyphenols content as gallic acid concentration [39]. Also, for the tea samples, the FC method was adopted by performing some changes in the protocol. In this case, 1 mL of tea extract has been diluted 10–75 times with deionized water, to be included in the calibration analytical range. It was then mixed with 1 mL of 3-fold-diluted FC phenol reagent and 2 mL of 35% sodium carbonate, shaken thoroughly and diluted

to 6 mL with water. The mixture is allowed to stand for 30 min to form the blue colored compound and its absorbance was measured at 700 nm [40].

3. Results and Discussion

3.1 Electrochemical and SEM characterization of MWCNTs-COOH/ ChAARTILs modified electrodes

All MWCNTs-COOH/ ChAARTILs modified electrodes were characterized by performing cyclic voltammetry (CV) experiments at different scan rates between 5 and 1000 mV s^{-1} in 1 mM $Fe(CN)_6^{3-/4-}$ (data not shown) in order to calculate electroactive area (A_{EA}, cm^2), heterogeneous electron transfer rate constant (k^0, cm s^{-1}) and the roughness factor (electroactive/geometrical area ratio, ρ). The A_{EA} has been evaluated using the Randles-Sevcik equation by plotting the peak current vs. square root of scan rates ($v^{1/2}$) [41]. k^0 was calculated using the extended method which merges the Klingler-Kochi and Nicholson-Shain methods for totally irreversible and reversible systems, respectively [42]. The A_{EA}, k^0 and ρ values for all the MWCNTs/ChAARTILs modified electrodes are reported in table 1. The obtained values for the modified electrodes are compared with those regarding an electrode modified with a well-known RTIL, namely [Bmim][NTf_2], in order to highlight the advantageous properties of cholinium-amino acids based RTILs for electrochemical platform development.

From the data reported in table 1, it is possible to observe an increase in terms of A_{EA}, ρ and k^0 placing MWCNTs on top of the electrode due to the efficient nano-structuration of the electrode [43]. Nevertheless, since MWCNTs tend to form bundles with a random organization onto the electrode surface, RTILs can play a key role in the organization through hydrogen bonding and π-π interactions [44]. MWCNT can interact with both cations cholinium or imidazolium forming fine bundles that can physically cross-link via cation-π or π-π interaction respectively [45]. Moreover, the different amino acid side chains seem to influence the MWCNTs bundles organization. In particular, [Ch][Gly] and [Ch][Ala] do not show any particular effect on A_{EA}, probably because of their simple side chains (-H, $-CH_3$) . With respect to the k^0 values, the [Ch][Gly] and [Ch][Ala] show an opposite behaviour by comparison with the MWCNTs modified GCE, [Ch][Ser] and [Ch][Cys] show a remarkable increase of A_{EA} and k^0 due to their side chains polar functional groups (-OH, -SH) . Finally, [Ch][Phe] exhibits the best performance in terms of A_{EA} (3.432 cm^2) and k^0 ($4.71 \cdot 10^{-3}$ cm s^{-1}) thanks to the homogeneous distribution of MWCNTs bundles onto the electrode surface, promoted by π-π interactions [46]. As an example in figure 1, the CVs at 50 mV s^{-1} for all the RTILs investigated are reported, it is possible to observe that MWCNTs-[Ch][Phe] shows the best performance.

In order to prove our hypothesis, the modified electrode with MWCNTs-[Ch][Phe] was further characterized by using scanning electron microscopy (SEM), as shown in figure 2 A-D. From the SEM picture reported in figure 2A, it is possible to see a homogeneous distribution of MWCNTs bundles which could be attributed to cation-π and π-π interactions between RTIL ions and MWCNTs surface [47, 48], Moreover, to validate our thesis, the elemental maps of the modified electrode were performed and are reported in figure 2 B-D. It is possible to see the homogeneous distributions of carbon, nitrogen, and oxygen, confirming a homogeneous distribution of MWCNTs onto the electrode surface, which could be beneficial for electrochemical biosensor development.
Therefore, we decided to further modify the GC/MWCNTs/[Ch][Phe] electrode with a model enzyme, namely *Trametes versicolor* laccase (*TvL*), to unequivocally prove the beneficial contribution of new cholinium-amino acids based RTILs on the enzyme immobilization. Finally, the modified electrode was tested for the determination of total polyphenol index [49].

3.2 Kinetic characterization of GCE/MWCNTs/[Ch][Phe]TvL biosensor

In order to assess the catalytic properties of GCE/MWCNTs/[Ch][Phe]/*Tvl* biosensor, CVs with GCE/MWCNTs/[Ch][Phe] and GCE/MWCNTs/[Ch][Phe]/*TvL* were performed in presence of gallic acid 0.2 mM in 50 mM acetate buffer pH 5 at scan rate of 5 mV s^{-1}, as shown in figure 3. The graph shows how the RTIL increases the electrocatalytic current, thanks to their ability to promote the enzyme entrapment [45, 50-53]. The CV of GCE/MWCNTs/[Ch][Phe] in gallic acid showed an electrochemical irreversible reaction. In presence of immobilized *Trametes versicolor* laccase onto the modified electrode, a cathodic current density of -20 µA cm^{-2} at -0.2 V vs. SCE (figure 3, inset) was observed. At the same time, the anodic peak significantly decreased according to the catalytic reaction pathway.

After a preliminary electrochemical characterization of GCE/MWCNTs/[Ch][Phe]*TvL* biosensor, the K_M^{app} and J_{max} values were determined by amperometric measurements in presence of gallic acid as substrate (S_{ox}). The kinetic parameters were obtained with the Michaelis-Menten approach and Lineweaver-Burk linearization (equations 1 and 2):

$$J_{lim} = \frac{J_{max}[S_{ox}]}{[S_{ox}]+K_M^{app}} \qquad (1)$$

$$\frac{1}{J_{lim}} = \frac{1}{J_{max}} + \frac{K_M^{app}}{J_{max}[S_{ox}]} \qquad (2)$$

where [S_{ox}] = oxidized substrate concentration, J_{lim} = cathodic current, K_M^{app} = apparent Michaelis-Menten constant, J_{max} = steady-state current [54, 55]. The kinetic and analytical performances of the developed biosensor are summarized in table 1SM.

Three separate electrodes were modified and tested as working electrodes, they showed a low variability of responses and a good repeatability (n= 10, 5 - 6 % error). Furthermore, a calibration curve for the GCE/MWCNTs/[Ch][Phe]/*TvL* biosensor has been obtained by using FIA analysis, as reported in figure 4A. The proposed biosensor was tested by injecting increasing gallic acid concentrations in the range between 0.006 and 10 mM (figure 4B), showing a linear range from 0.006 up to 0.3 mM, as reported in the inset of figure 4B.

Afterwards, the lifetime of the GCE/MWCNTs/[Ch][Phe]/*TvL* biosensor has been evaluated by injecting 0.2 mM gallic acid over 20 days showing a decrease of 10% after the first 10 days, reaching 33% after 20 days, as shown in figure 4C. These results can be ascribed to the anti-fouling properties of RTILs, high enzyme loading, tight enzyme immobilization retaining its native structure and biological activity.

Finally, the performance of the proposed GCE/MWCNTs/[Ch][Phe]/*TvL* biosensor has been compared with other electrochemical platforms showing a remarkable increase of the current density maximum, high sensitivity, and good linear range, as reported in table 2 [56-58].

3.3 Polyphenol analysis in wine and tea samples

The proposed biosensor was used to quantify total polyphenols content in three real samples: a white wine, a red wine, and a black tea. The obtained results (three replicates of each sample) were compared with spectrophotometric measurements performed with the Folin-Ciocalteau method (table 2SM). The values obtained with the biosensor are in good agreement with the reference method and show a recovery range between 90 and 98 %. This discrepancy is probably due to the low specificity of the Folin-Ciocalteau method, where other species could be oxidized in addiction to polyphenols, giving overvalued results [59]. Indeed, by using the biosensing method, there is no interference due to reducing sugars. In the experimental conditions used, sugars are not electroactive. For the same reason, in order to avoid the possible interference of other reducing agents, such as ascorbic acid or quercetin, the analyses were performed at a low potential,-100 mV vs SCE, [60].

4. Conclusions

The experimental results herein shown suggest the potentiality of ChAARTILs as immobilization matrix for the development of electron transfer-based biosensors. By exploiting a wide range of

amino acids, we are able to synthesize several ChAARTILs, that can be used to construct electrochemical biosensors with different analytical characteristics.

The proposed MWCNTs/[Ch][Phe] combination showed several peculiar features: a) homogeneous distribution of MWCNTs bundles; b) efficient entrapment of proteins without loss of bioelectrochemical properties, suggesting the preservation of the native-like structure; c) high electron transfer efficiency due to the interactions occurring between carbon nanotubes and room temperature amino acids based ionic liquids.

Moreover, the proposed biosensor can be easily prepared by simple layer-by-layer modification of the working electrode. It showed a low detection limit (3 µM) and a wide linear range (0.006-0.3 mM) for polyphenols determination in beverages. Furthermore, it provides a significant increase in sensitivity with respect to other enzymatic biosensors reported in the literature. The developed biosensor shows a fair stability, losing only 30 % of response along 20 days. Is has to be considered also that the CHAARTILs are eco-friendly, avoiding the use/production of hazardous compounds.

Therefore, the proposed GCE/MWCNT/[Ch][Phe]/*TvL* biosensor can be efficiently used as disposable device for the evaluation of polyphenol index in real samples.

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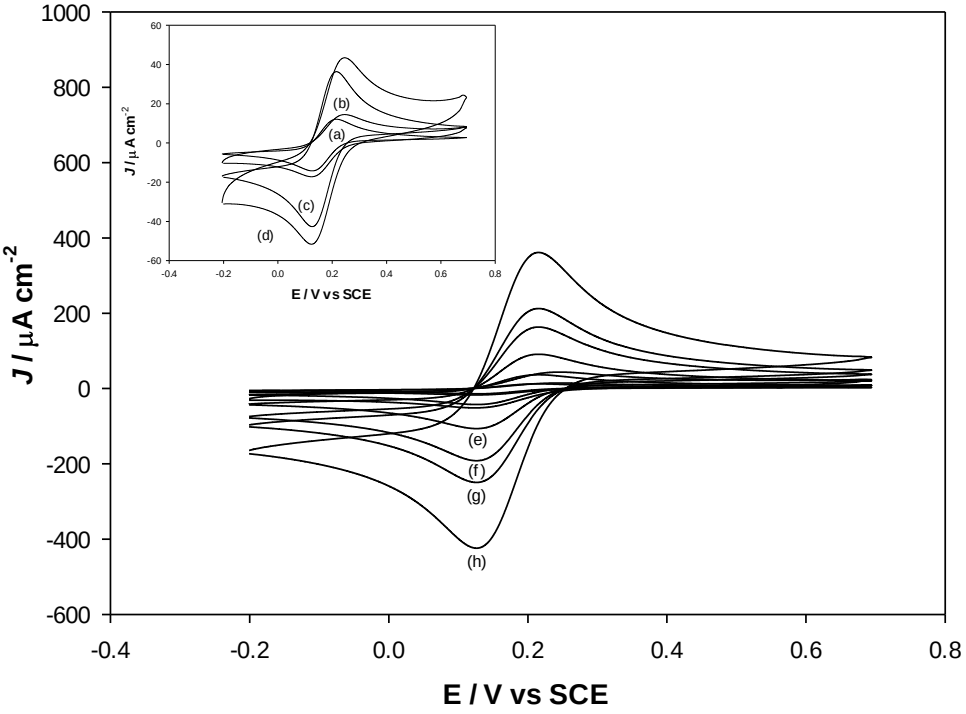


Figure 1 CVs recorded in 1 mM $K_3Fe(CN)_6$ in 50 mM PBS buffer, pH 7 at v=50 mVs^{-1} with (a) GCE; (b) GCE/MWCNTs/[Ch][Ala]; (c) GCE/MWCNTs; (d) GCE/MWCNTs/[Ch][Gly]; (e) GCE/MWCNTs /[BMIM][NTf_2]; (f) GCE/MWCNTs/[Ch][Ser]; (g) GCE/MWCNTs/[Ch][Cys]; (h) GCE/MWCNTs [Ch][Phe].

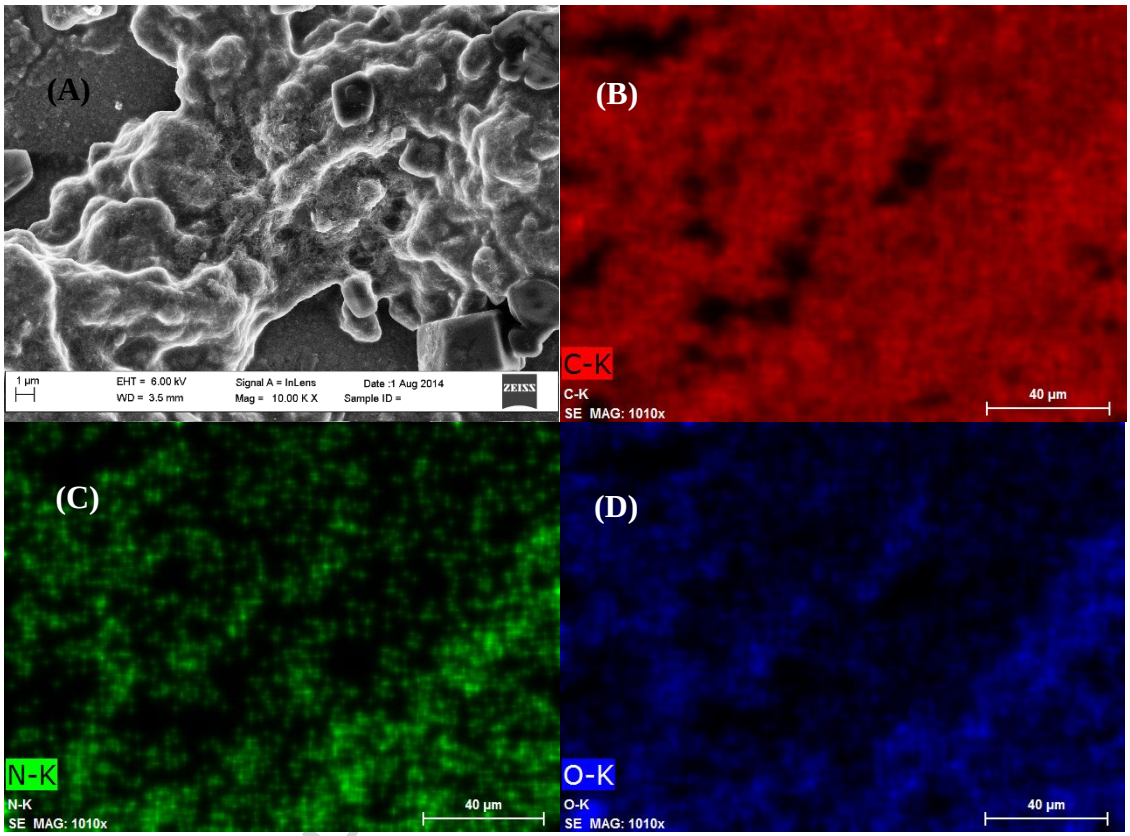


Figure 2 SEM picture of GCE/MWCNTs/[Ch][Phe] (A) and elemental map analysis of carbon (B), nitrogen (C) and oxygen (D).

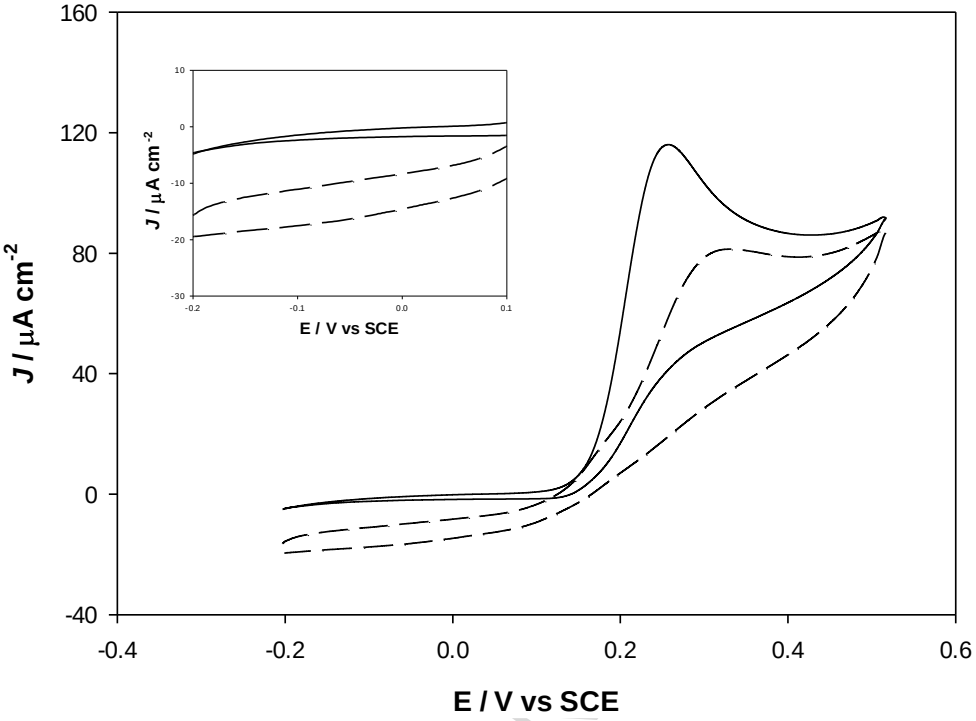


Figure 3 CVs for gallic acid 0.2 mM recorded with GCE/MWCNTs/[Ch][Phe]/*Tv*l (dashed line) and GCE/MWCNTs/[Ch][Phe] (solid line) in 50 mM acetate buffer, pH 5 at scan rate of 5 mVs^{-1}. Inset: details of current at -100 mV vs SCE.

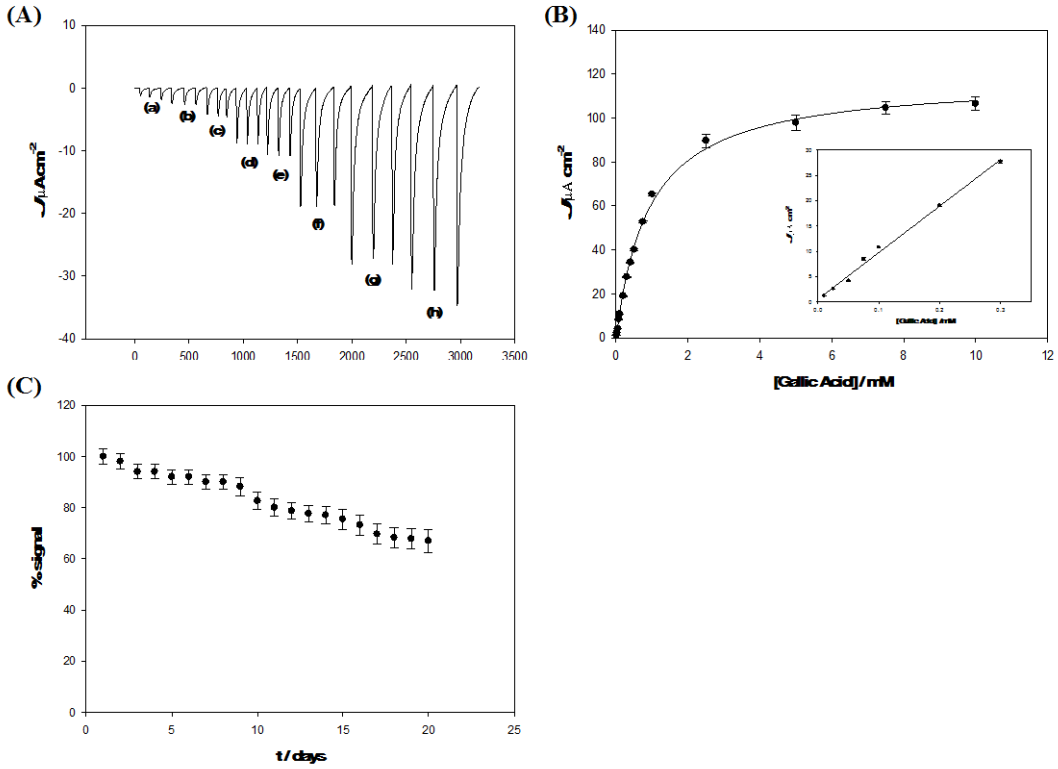


Figure 4 (A) Chronoamperogram of FIA experiments with GCE/MWCNTs/[Ch][Phe]*Tvl* reporting current density with respect to time for different concentration of gallic acid: (a) 0.006 mM; (b) 0.012 mM; (c) 0.025 mM; (d) 0.050 mM (e) 0.075 mM; (f) 0.1 mM; (g) 0.2 mM; (h) 0.3 mM in 50 mM acetate buffer, pH 5, at - 0.1 V vs SCE; **(B)** Current density of GCE/MWCNTs/[Ch][Phe]/*Tvl* electrode with respect to the gallic acid concentration (0.006-10 mM) in 50 mM acetate buffer, pH 5, at - 0.1 V vs SCE. Inset: linear response for the low concentrations range; **(C)** Lifetime of biosensor in the presence of 0.2 mM gallic acid solution in 50 mM acetate buffer, pH 5, at - 0.1 V vs SCE.

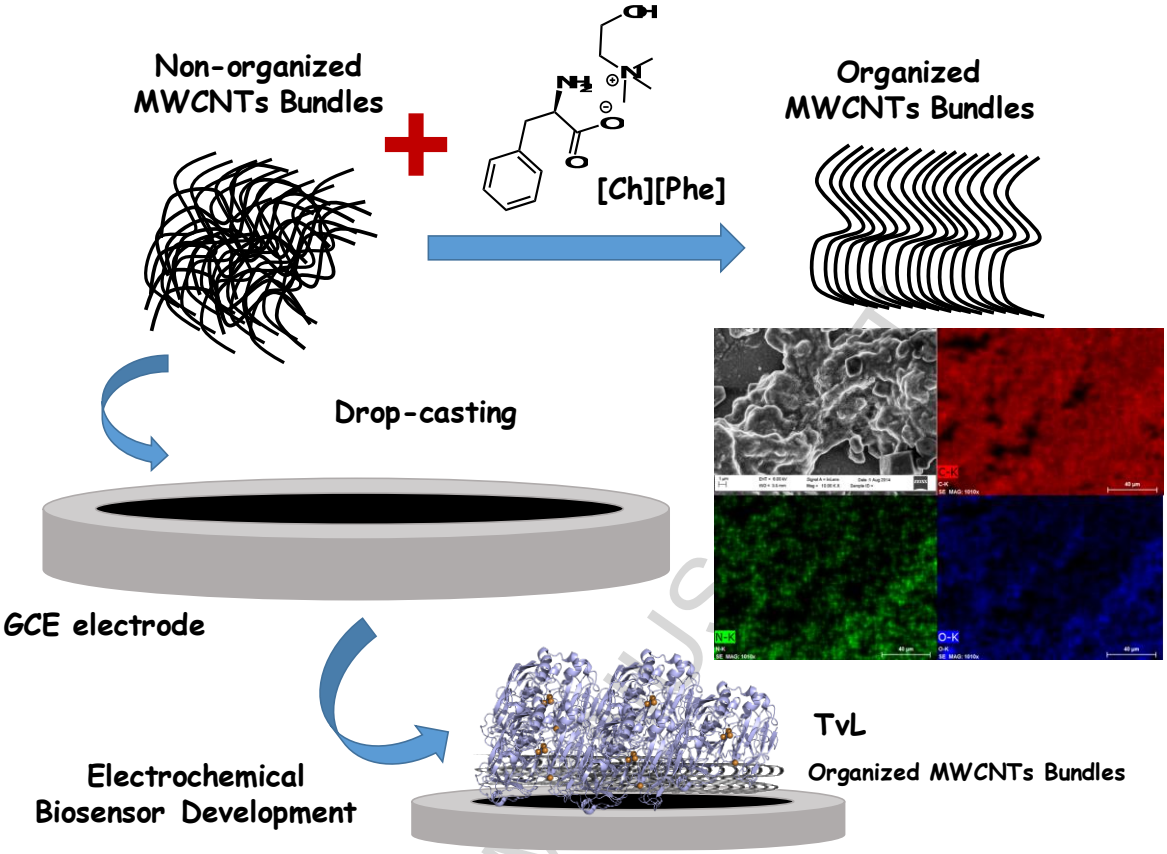
	A_{ea} (cm^2)	k^0 (10^{-3} cm s^{-1})	ρ
GCE	0.07 ± 0.01	0.31 ± 0.09	0.99 ± 0.01
GCE/MWCNT	0.19 ± 0.01	1.35 ± 0.17	2.69 ± 0.01
GCE/MWCNT/[Ch][Ala]	0.17 ± 0.01	0.49 ± 0.12	2.41 ± 0.01
GCE/MWCNT/[Ch][Gly]	0.22 ± 0.01	2.93 ± 0.35	3.12 ± 0.01
GCE/MWCNT/[Ch][Ser]	1.06 ± 0.10	4.35 ± 0.42	15.01 ± 0.10
GCE/MWCNT/[Ch][Cys]	2.10 ± 0.25	4.43 ± 0.54	29.75 ± 0.25
GCE/MWCNT /[Ch][Phe]	3.43 ± 0.36	4.71 ± 0.36	48.58 ± 0.36
GCE/MWCNT/[Bmim][NTf_2]	0.63 ± 0.04	3.62 ± 0.22	8.92 ± 0.04

Table 1 Electrochemical parameters for different modified electrodes. GCE = glassy carbon electrode, MWCNT = multi-walled carbon nanotubes, [Ch][AA] = generation IV ionic liquid choline and amino acid based, [Bmim][NTf_2] = generation II ionic liquid.

Electrochemical platform	**Appl. Pot. (mV) vs SCE**	**K_m^{app} (mM)**	**J_{max} (μA cm^{-2})**	**Sensitivity (μA mM^{-1} cm^{-2})**	**LOD (mM)**	**Linear range (mM)**
***TvL*/Full-AuNPs/SAM-AuE°**	-150	0.66	36.2	72.4	6 10^{-3}	0.03-0.3
Nafion/Lac/Sonogel-Carbon§	-150	0.076	-	-	0.41 10^{-3}	0.0001-0.022
TvL*/PAP/MWCNT-SPE	-150	0.81	20.7	12.2	0.6 10^{-3}	0.0006–0.1
MWCNTs/[Ch][Phe]/*TvL* (this work)	-100	0.92	118.0	91.9	3 10^{-3}	0.006 – 0.3

Table 2 Amperometric biosensors based on different Laccase for determination of polyphenol index in beverages (data from: ° ref. 56; § ref. 57; * ref. 58). Abbreviations: gold nanoparticles (AuNPs), gold electrode (AuE), fullerenol (Full), self-assembling monolayer (SAM), *Trametes versicolor* laccase (TvL), laccase (Lac), multiwalled carbon nanotubes (MWCNTs), screen printed electrode (SPE), cholinium ion (Ch), phenylalanine (Phe).

Graphical Abstract



Highlights

- The new room temperature ionic liquids (RTILs) are based on choline and different amino acids.
- RTILs are efficient immobilization matrix for enzymes and nanomaterials.
- The biosensor based on *TvL* has good performances and high stability in terms of life-time.
- The amperometric biosensor developed is able to detect total polyphenols content.