Silver release from polypyrrole matrix in well water

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ABSTRACT

Polypyrrole (PPy) films constituted by hollow rectangular microtubes were electrosynthesised from a salicylate (Sa) solution on 316L stainless steel (316L SS) electrodes. The covered electrodes were dipped in AgNO$_3$ solution achieving the immobilisation of silver species. After this, they were rotated at different speeds and silver transport from the PPy matrix to well water under open circuit potential (OCP) condition was studied. The results indicate that the diffusion process of silver from the polymeric matrix is accelerated when the electrode speed is increased. The disinfection process of well water contaminated with *Escherichia coli* was also analysed for the electrode covered with PPy/Ag films. A faster release of Ag species was obtained when a potential step was applied. The microtubular morphology of the polymer does not suffer any alteration during the experiments.

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1. Introduction

Materials with antibacterial activity have been intensively investigated in the latest years. Different biomaterials with bactericidal species have been employed in fields such as medicine, dentistry and water treatment [1,2]. Recently, it was informed that 502,000 diarrhoea deaths were estimated to be caused by inadequate drinking water in 2012 [3]. According to this, it is essential to provide a safety environment, protect the human health and remove the pathogens from drinking water. Nowadays, in water treatments there is a challenge to find a disinfection system to avoid harmful by-products generated from using conventional chemical disinfectants [4]. The non-conventional antimicrobial compounds most frequently used in water disinfection are chitosan, carbon nanotubes and silver species [2]. Since ancient times, the silver species have been known to be effective against a broad range of microorganisms [5,6]. There are various mechanisms by which silver species affect the cell functionality. Some authors have shown that Ag$^+$ interact with thiol groups in proteins, resulting in inactivation of respiratory enzymes and leading to the production of reactive oxygen species (ROS) [7]. On the other hand it was also proposed that silver ions disrupt the deoxyribonucleic acid (DNA) replication and damage the cell membrane [8]. In recent years, silver species have been incorporated into different materials such as polymers [9] and silica composites [10] for water treatment applications. A material with antimicrobial activity employed in water disinfection not only requires antimicrobial capacity but also mechanical properties and flow resistance. Recently, some authors showed effective water filters constituted by silica [10] or alginate beds [11] containing Ag nanoparticles. Both filters exhibit a high disinfection performance in water contaminated with *Escherichia coli*.

On the other hand, the synthesis of polypyrrole-based micro- and nanostructures has become a topic of increasing interest due to their several applications [12,13]. One of the most interesting morphological features of these microstructures is a large surface area and the possibility to be useful as drug reservoirs. The active agents can be entrapped during the electropolymerisation in the polymeric matrix or they can be immobilised later [14,15].

Drug release can be carried out by simple immersion of the polymer-coated material. A faster rate of release can be obtained when an electrical stimulation such as a potential step is applied.

In a previous study it was demonstrated that the electrosynthesis of polypyrrole (PPy) in aqueous solutions of sodium salicylate (NaSa) leads to the formation of hollow rectangular-sectioned microtubes onto 316L stainless steel (316L SS) [16,17]. These microstructures were used to immobilise Ag and the modified electrode presented a very good performance towards inhibition of *Staphylococcus aureus* bacteria activity [18]. It was proposed that Ag immobilisation in the polypyrrole matrix occurs in two stages. Initially, when the electrode is immersed into the AgNO$_3$ solution, Ag$^+$ ions are concentrated in the film due to the high content of salicylate anions. Then, some of these cations can be reduced while non-oxidised segments within the polymer are oxidised. Therefore, both Ag$^+$ ions and metallic Ag are present in the PPy matrix.

It is of great importance to find a well water disinfection system which does not produce harmful by-products. In this way the Ag release from polymer surface forbid the by-product toxicity. There are several methods to modify the material surface with silver particles [19,20]

and it is preferable to use a simple technique. The aim of this work was to evaluate the capacity of PPy/Ag films deposited onto 316L SS for the decontamination of well water. The effect of hydrodynamic conditions on the silver release from the PPy matrix was analysed. Lastly, the antimicrobial activity of covered PPy/Ag films against *E. coli* ATCC 25922 in contaminated well water was evaluated. *E. coli* is considered the best indicator of health risk from drinking and recreational water.

2. Experimental

The working electrode (WE) was a rod (3 mm in diameter) of 316L SS (wt.% is: 17.47 Cr, 10.32 Ni, 1.88 Mn, 1.90 Mo, 0.39 Si, 0.025 C and Fe balance) embedded in a Teflon holder. The exposed surface area of the WE is 0.07 cm^2. Before each experiment, the surface was abraded to a 1200 grit finish using SiC, then degreased with acetone and finally washed with triply distilled water. All the potentials were measured against Ag/AgCl (3 M KCl) reference electrode and a platinum sheet was used as a contraelectrode. The films were obtained potentiostatically at 0.80 V during 600 s from a solution containing 0.25 M pyrrole (Py) and 0.5 M NaSa. After PPy electrodeposition, the electrode was intensively washed with distilled water and dipped in a 0.05 M AgNO$_3$ solution during 8 h under open circuit potential (OCP) in dark conditions.

Electrochemical studies were carried out with a potentiostat/galvanostat Autolab/PGSTAT 128N. Morphological studies of films metalised with gold were performed by scanning electron microscopy (SEM) ISI DS 130 coupled with an energy dispersive X-ray (EDX) analyser model EDAX 9600. Silver release experiments were carried out in 30 mL of well water using a rotating disc electrode EDI 101 (Radiometer Analytical S.A.) with a CTV 101 rotation rate controller (Radiometer Analytical S.A.). The well water composition is presented in Table 1.

The antibacterial activity of the PPy/Ag films under hydrodynamic conditions was determined against a reference strain of *E. coli* ATCC 25922. For the experiments, a loop of frozen cells stored at −70 °C in a tripticase soy broth (TSB) (Biokar) supplemented with 20% v/v glycerol (Biopack) was grown in nutrient rich broth (Britania) for 24 h at 37 °C. Then, the cells were collected by centrifugation at 5000 rpm for 10 min, washed twice with distilled sterile water and diluted with sterile well water to give a working culture of approximately 10^5 CFU (colony forming units mL^{-1}) (N$_o$). Thirty millilitres of this cell suspension in well water was used for testing each hydrodynamic condition during 8 h at 20 °C. To determine the number of cells remaining in each treatment aliquots of 1 mL were taken every 2 h (N$_t$), serially diluted tenfold and the resulting dilutions plated onto plate count agar (PCA) (Britania) incubated at 37 °C for 24 h.

The bactericidal activity (BA) was calculated as follows:

$$BA\% = [(N_0 - N_t)/N_0] \times 100.$$

Released silver concentration in well water from PPy/Ag-coated 316L SS samples was determined using inductively coupled plasma atomic emission spectrometry (ICP-AES).

Table 1
Well water composition.

Element	Concentration (mg L^{-1})
Na	356
Mg	28.5
Ca	95.8
S (sulphate)	86.5
P (phosphate)	0.151
Cl (chloride)	408.5
pH	7.7

3. Results and discussion

Electropolymerisation of Py onto 316L SS was performed in 0.50 M Sa and 0.25 M Py at 0.80 V during 600 s. In our previous work it was demonstrated that under this experimental condition the PPy film morphology is constituted by hollow rectangular-sectioned microtubes [16,17].

After the polymer synthesis, the covered electrode was dipped in a 0.05 M AgNO$_3$ solution during 8 h under OCP conditions. The measured potential was 0.46 V vs. Ag/AgCl during all the experiences which correspond to the potential of the Ag$^+$/Ag redox pair for a 0.05 M AgNO$_3$ solution [18]. Differences between concentrations of Ag determined by ICP-AES before and after immersion allowed that the estimated amount of silver species incorporated into the film was 2 mg.

SEM micrograph and EDX analysis of the electrode surface are presented in Fig. 1. The surfaces of the microtubes are covered randomly by rosette-like aggregates with a highly rough surface and the EDX analysis shows the Ag signal, which indicates the effective immobilisation of silver species.

The electrodes covered with PPy/Ag were rotated at different speeds during 8 h in the well water and the OCP was measured (Fig. 2). For comparative purposes the curve corresponding to the uncoated electrode is also presented (Fig. 2, curve e). The OCP vs. time curves
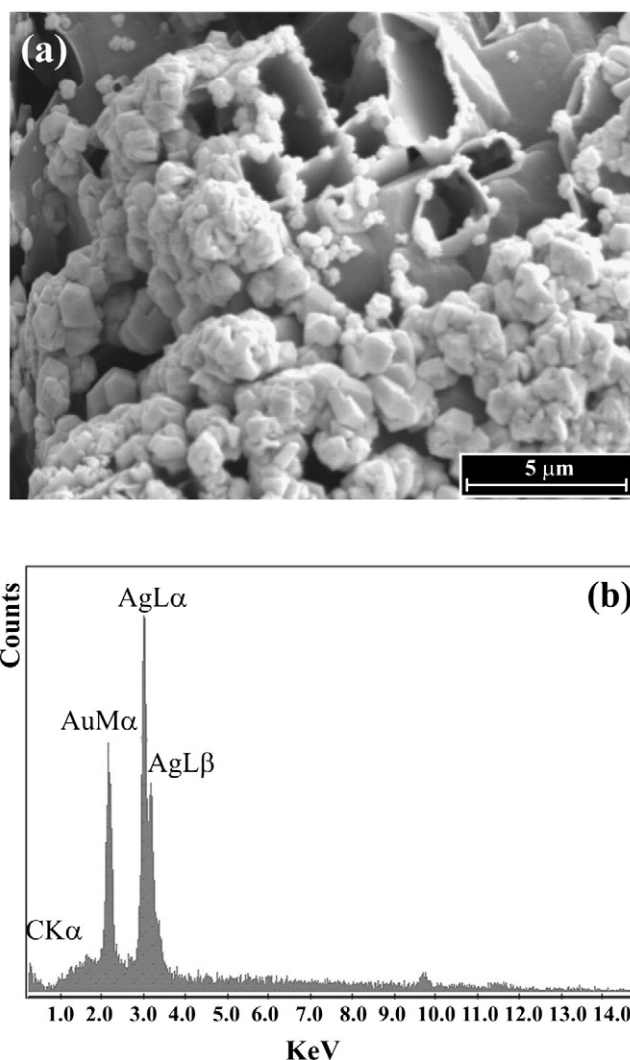

Fig. 1. (a) SEM image and (b) EDX analysis of PPy-coated steel electrode after immersion during 8 h in 0.05 M AgNO$_3$ under open circuit conditions. The film was formed potentiostatically at 0.80 V vs. Ag/AgCl during 600 s in 0.5 M Sa solution containing 0.25 M Py.
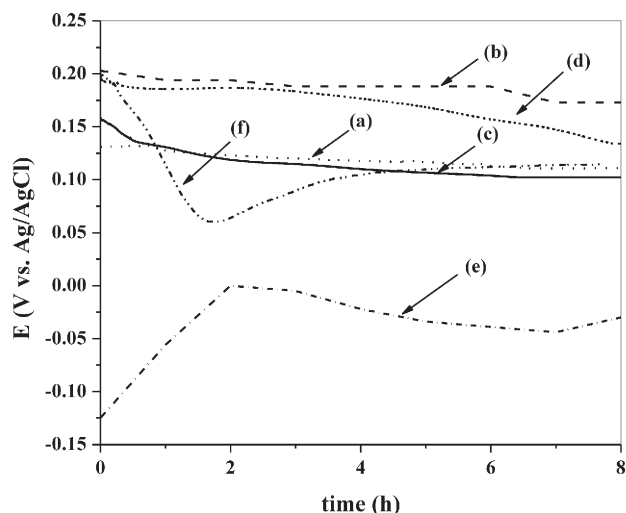
Fig. 2. OCP vs. time plot in well water for PPy/Ag-coated steel electrodes rotated at: (a) 0, (b) 1000, (c) 3000 and (d) 5000 rpm. The results for the static uncoated and PPy-coated steel electrodes are also included (curves e and f, respectively).
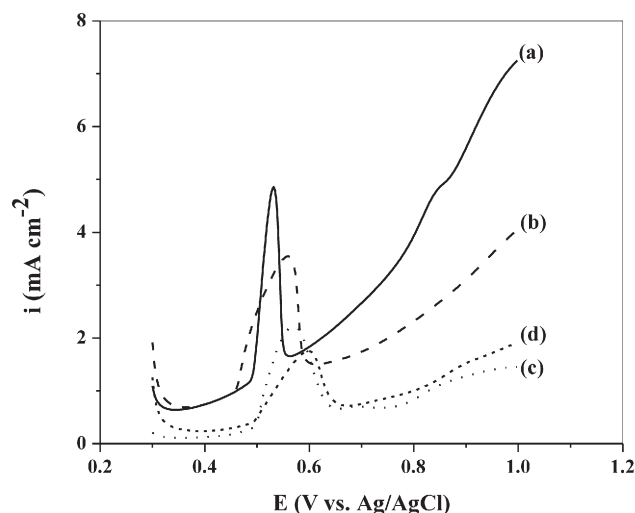

Fig. 3. Anodic polarisation curves registered in 0.5 M Sa at 0.001 V s^{-1} for PPy/Ag-coated steel electrodes after being rotated in well water during 8 h at: (a) 0, (b) 1000, (c) 3000 and (d) 5000 rpm.

are not highly reproducible probably due to the difference between the exposed surfaces of the microtubes. In spite of this, the OCP values of the electrodes covered with PPy/Ag films fall always between 0.1 and 0.2 V. The stabilised OCP values for a static electrode coated with the unmodified polymer also lie within this potential interval (Fig. 2, curve f). Thus, the presence of the coatings causes an anodic shift of the corrosion potential which suggests an anodic protection mechanism and an effective physical barrier against corrosion.

An ICP-AES analysis was carried out in order to evaluate the influence of the hydrodynamic conditions on the amount of Ag species released. The results obtained after OCP measurements under different rotation speeds are presented in Table 2. The total amount of silver species in well water corresponds to colloidal Ag particles and Ag$^+$ ions. It can be observed that an increment of silver release in the well water as the rotation speed increases and the measured concentrations are below the maximum recommended level of silver in drinking water (0.1 mg L^{-1}) [21,22].

With the purpose to check the amount of residual metallic silver immobilised in the films after the releasing experiments, an anodic polarisation in 0.5 M Sa solution was carried out for all the coated samples (Fig. 3). In all the cases a strong well-defined anodic peak at 0.50 V is observed which is associated to the one electron oxidation of metallic silver [18]. At more positive potential a current increase due to polymer oxidation is registered. As the rotation speed increases the charge corresponding to the silver oxidation peak decreases. These results demonstrated that the hydrodynamic conditions affect the amount of silver released from the PPy coating.

The PPy/Ag-coated steel electrodes were analysed by SEM/EDX after immersion under OCP condition in the well water during 8 h (Fig. 4). In all the cases, there is no observable damage in the PPy microtubes despite the hydrodynamic conditions. This result together with the

measured OCP values would indicate that PPy/Ag films are able to protect the stainless steel even under hydrodynamic conditions over a relatively prolonged period of time.

SEM examination of the non-rotating electrode shows that the microtubes are surrounded by rosette-like aggregates (Fig. 4(a)). On the other hand, the morphology of the deposits changes as the rotation speed increases. When the PPy/Ag covered electrode was rotated at 3000 rpm, the coexistence of noodle-like microstructures with the rosette-like aggregates is observed (Fig. 4(b)). There are no rosette-like aggregates found on the surface of PPy/Ag when the electrode was rotated at 5000 rpm (Fig. 4(c)). The surface of Ag-modified PPy was totally covered by noodle-like microstructures. The formation of these microstructures is clearly associated with the high rotational speed.

After immersion under stagnant condition the EDX spectrum indicates not only the presence of Ag but also of Cl in the sample analysed (Fig. 5(a)) This result is associated with the formation of AgCl due to the presence of chloride ions in the well water. On the contrary, the EDX analysis does not show the signal of chloride for the highest rotation speeds (Fig. 5(b) and (c)). In this case, the formation of a white precipitate in the well water was observed after the experiments.

In order to obtain more information about the Ag release in the well water, a potential step from 0 to 0.50 V vs. Ag/AgCl was applied. Fig. 6 represents the chronomperometric curves for 316L SS/PPy systems unmodified and modified with silver. The measured current density (0.07 mA cm^{-2}) for unmodified 316L SS/PPy system (small insert in Fig. 6) is independent of the rotation rate and it is related with the oxidation of the polymer. Higher current densities were measured for the electrode covered with the modified polymer due to the dissolution of deposited Ag (small insert in Fig. 6). The shape of the *i* vs. *t* transients indicates that Ag oxidation took place simultaneously with polymer oxidation. The figure also shows the influence of the electrode rotation speed (ω) on the current transients corresponding to the modified 316L SS/PPy system. As the rotation speed increases the maximum current density involved during the oxidation reaction of Ag also increases in the range between 0 and 1000 rpm. The linear dependence of the maximum current density with the square-root of rotation speed ($\omega^{1/2}$) (Fig. 7) indicates that the oxidation process is controlled by diffusion of Ag$^+$ ions in the bulk solution. A subsequent increase in the rotation speed value in the range of between 2000 and 5000 rpm does not lead to an increase in the maximum current density, which
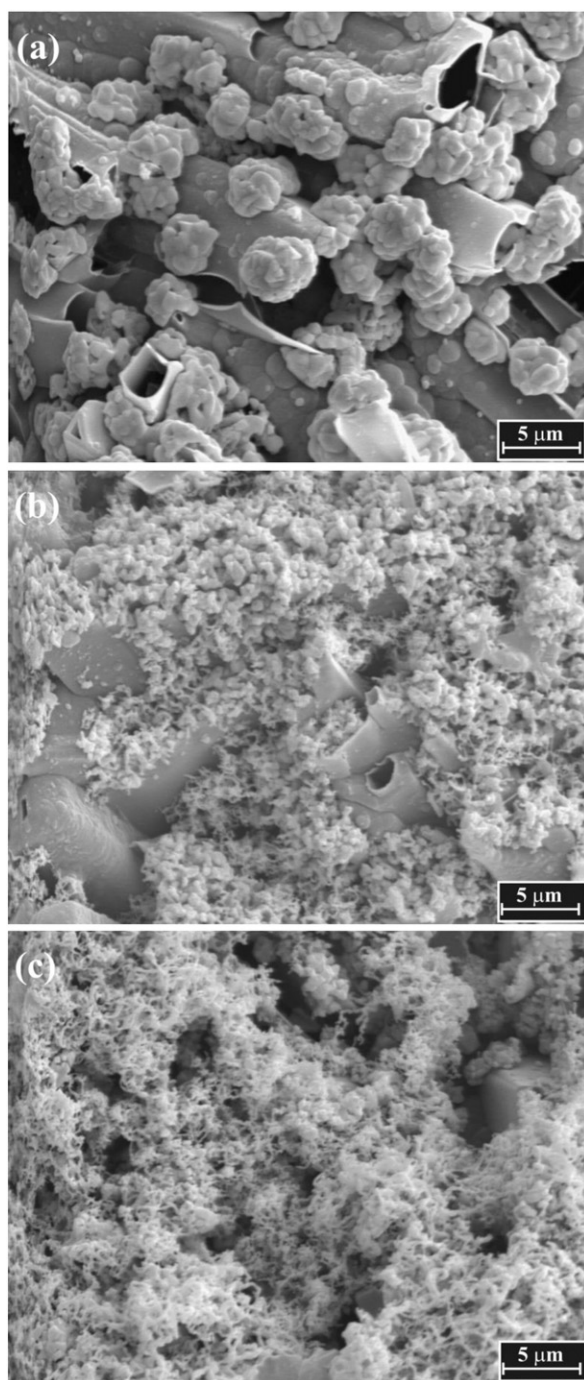
Table 2
Concentration of Ag released in the well water samples for the PPy/Ag-coated steel electrodes under OCP conditions at different rotation speeds.

Speed rotation (rpm)	Concentration (mg L^{-1})
0	0.020
1000	0.053
3000	0.090
5000	0.093

Fig. 4. SEM images of PPy/Ag-coated steel electrode after being immersed in well water during 8 h at: (a) 0, (b) 3000 and (c) 5000 rpm.

Fig. 5. EDX examination of the PPy/Ag-coated steel electrode after immersion in well water during 8 h and under OCP conditions at: (a) 0, (b) 3000 and (c) 5000 rpm.

indicates that the process is no longer controlled by the diffusion of Ag^+ ions in the well water. Probably, the process is controlled by the diffusion of Ag species in the film.

The charge associated with silver oxidation is independent of the electrode rotation speed (Fig. 6). On the other hand, the 316L SS/PPy/Ag system reaches the base value of current density corresponding to the oxidation of the polymer in a shorter time as the rotation speed increases. By subtracting the current density corresponding to PPy oxidation, the charge involved in the oxidation process of silver can be determined by integrating the area under the i vs. t curve. A value of 0.56 C cm^{-2} was obtained independently of ω value which corresponds to an amount of 0.04 mg of immobilised metallic Ag in the PPy matrix. According to this, only the 2% of the total amount of immobilised species
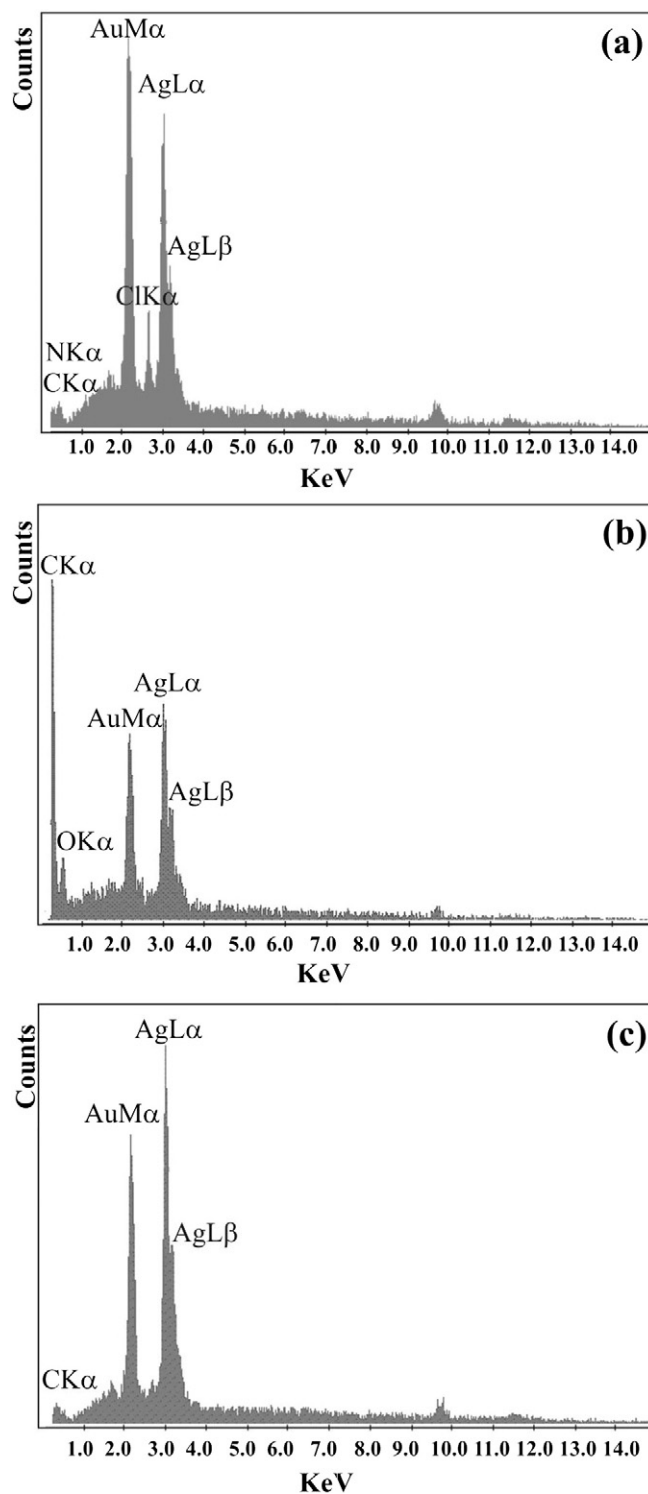
is oxidised. In addition, the released concentration of Ag^+ ions can be estimated to be 1.083 mg L^{-1}, which is as expected much greater than the amount released under OCP conditions during 8 h.

On the other hand the antibacterial activity of the PPy/Ag films under hydrodynamic conditions against *E. coli* ATCC 25922 was investigated. Fig. 8 shows the survival changes of *E. coli* as a function of time. As seen in the figure, when the rotational speed is increased, the number of cells of *E. coli* in the well water decreases.
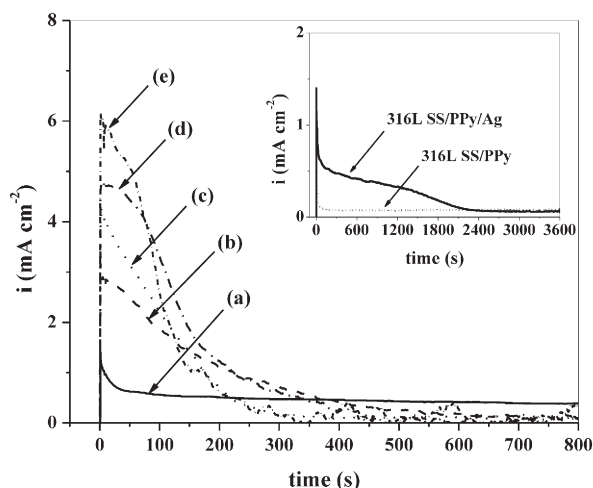
Fig. 6. Anodic current transients obtained in well water at 0.50 V vs. Ag/AgCl for PPy/Ag-coated steel electrode at different rotation speeds: (a) 0, (b) 250, (c) 500, (d) 750 and (e) 1000 rpm. Small insert: anodic current transients obtained under static condition for 316L SS/PPy and 316L SS/PPy/Ag system at 0.50 V vs. Ag/AgCl.

The maximum reduction in the cell number was 4 log units when the electrode was rotated at 5000 rpm for 8 h, which means a bactericidal effect of 99.99%. This effect was less evident without rotation, where after 8 h of assay a bactericidal effect of 93.50% was obtained..

Since the CFU mL^{-1} applied in this study were higher than those found in real-life systems and inhibition depends on the concentration of metallic Ag as well as on the CFU of bacteria used in the experiments [23], it appears that these particles could have an excellent biocide effect and effectiveness in reducing bacterial growth for practical applications such as the formulation of various biocidal materials. The antimicrobial activity of silver species against *E. coli* is also associated with the presence of AgCl particles [24]. Thus, in spite of precipitation of AgCl resulting in a decrease of Ag$^+$ ion concentration in the well water, the biocide effect is not adversely affected.

4. Conclusions

Silver species can be released from a PPy matrix constituted by hollow rectangular microtubes deposited onto 316L SS. The transport of silver species from the polymer surface to the well water was increased as the rotation speed was enhanced.
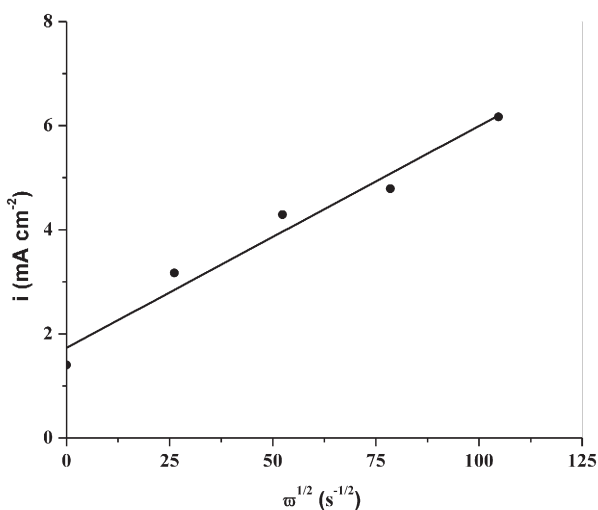

Fig. 7. Anodic peak current dependence with the square-root of rotation rate obtained from the results presented in Fig. 6.
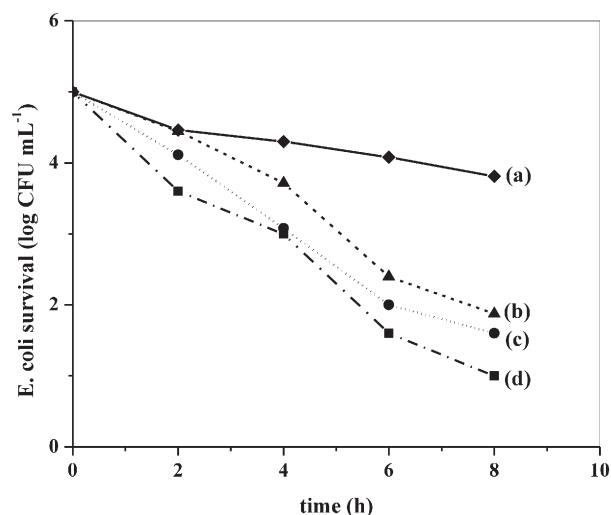

Fig. 8. Bactericidal activity of the PPy/Ag-coated steel electrode against *Escherichia coli* ATCC 25922 in well water rotated at: (a) 0, (b) 1000, (c) 3000 and (d) 5000 rpm.

The coatings showed a good anticorrosion performance in well water. The system also presented antibacterial activity which increases as the rotation rate is incremented. A faster release of Ag species was obtained when a potential step is applied.

The results of the present work may provide a novel approach for the design of antibacterial materials in water disinfection system.

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