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Biomimetic properties and surface studies of a piezoelectric caffeine sensor based on electrosynthesized polypyrrole

Benilda S. Ebarvia[a], Sharlene Cabanilla[b], Fortunato Sevilla III[a,b,c,*]

[a] Graduate School, University of Santo Tomas, España, Manila, Philippines
[b] College of Science, University of Santo Tomas, España, Manila, Philippines
[c] Research Center for the Natural Sciences, University of Santo Tomas, España, Manila, Philippines

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Abstract

An approach for preparing a chemical sensor for caffeine through the combination of molecularly imprinted polypyrrole and a piezoelectric quartz transducer was proposed. The caffeine-imprinted polymer was synthesized using galvanostatic electropolymerization of pyrrole monomer directly onto one of the gold electrodes of a 9 MHz AT-cut quartz crystal in the presence of caffeine. The optimum conditions for the electrosynthesis of the reagent phase were established. Caffeine molecules were entrapped in the matrix of polymer film, and were removed by subsequent washing with water, leaving behind pores capable of recognizing the target analyte molecule.

The caffeine sensor was fixed in a measuring cell and measurement of the resonant frequency of the quartz crystal as it comes in contact with the caffeine solution was carried out in a stopped flow mode. A steady-state response was achieved in about 10 min. The sensor exhibited a linear relationship between the frequency shift and the ln of caffeine concentration in the range of 0.1–10 mg/mL (correlation coefficient, $r = 0.9882$). The sensitivity of the sensor was about 255 Hz/ln concentration (mg/mL). A good repeatability, R.S.D. = 9 ($n = 6$) for 0.5 mg/mL caffeine solution was also observed. The use of the sensor can present a potential low-cost option for determining caffeine.

Surface analytical techniques such as scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) were performed on the polymer coating in order to elucidate the imprinting process and rebinding of caffeine to the polymer matrix during the sensing process. The SEM micrographs and XPS spectra revealed features and structures that could support the imprinting and recognition of caffeine molecule by the imprinted polymer.

Keywords: Biomimetic sensor; Molecularly imprinted polypyrrole; Caffeine sensor; Piezoelectric sensor; Galvanostatic polymerization

1. Introduction

The molecular recognition capability of molecularly imprinted polymers (MIPs) has been described in a wide range of applications [1–8] including chemical sensors, immunoassay, directed synthesis and enzyme and receptor binding. MIPs are smart synthetic materials that are utilized instead of natural biomolecules because of their high selectivity to target molecule. The difficulties encountered in handling biological materials (e.g. complications in its preparation with the desired properties and instability in high temperature and solvents) are avoided when MIPs are employed.

Recently, molecular imprinting has been approached using the electrosynthesis of conducting polymers through galvanostatic, potentiostatic and cyclic voltammetric methods [9–11]. These methods provide a simple and rapid technique of controlling the thickness of the conductive polymer film grown adherent to a transducer of any size and shape. The monomers that have been used in the design of molecularly imprinted conducting polymers include pyrrole, aniline and *o*-phenylenediamine.

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[*] Corresponding author. Present address: Industrial Technology Development Institute, Department of Science and Technology Bicutan, Taguig, Metro Manila, Philippines. Tel.: +63 7314031; fax: +63 7409730.
E-mail address: fsevilla@ustcc.ust.edu.ph (F. Sevilla III).

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In sensor applications, polypyrrole (PPy) is one of the most explored conducting polymer [12–17]. It has been employed in the reagent phase of a number of potentiometric, amperometric, conductimetric and optical sensors for chemical species. In this work, the feasibility of integrating molecularly imprinted electrosynthesized polypyrrole and the piezoelectric quartz crystal transducer is presented as an attractive strategy for the development of a chemical sensor for caffeine. The analytical performances of the biomimetic caffeine sensor based on PPy were characterized, together with the surface morphology and elemental composition of the molecularly imprinted PPy. Quantitation of caffeine was achieved through the change in the resonant frequency of the piezoelectric quartz crystal. This shift in frequency is correlated with the change in mass of the surface of the sensing layer upon contact with the target analyte.

The use of the designed sensor could present one possible alternate course for detecting caffeine in food, beverages and pharmaceutical products. It could also find important application in the assessment of environmental water quality, since caffeine has been recognized as a marker for the fecal contamination of water [18]. It offers a simple and inexpensive option for the measurement of caffeine, which is often analyzed using tedious procedures and costly bulky instruments, such as tandem chromatographs.

2. Experimental

2.1. Reagents and chemicals

All chemicals used were of analytical reagent grade, and all solutions were prepared with distilled water as the solvent. Pyrrole monomer was obtained from Sigma Chemicals Co. (St. Louis, MO, USA) and was distilled at 124 °C, flushed with nitrogen, and stored in amber bottles to prevent atmospheric oxidation. Caffeine, xanthine and theophylline were also purchased from Sigma Chemicals Co. and used as received. Phosphate buffer solution (0.1 M, pH 7) was used for all measurements and stored in the dark at 4 °C.

2.2. Sensor preparation

The piezoelectric caffeine sensor was prepared by direct deposition of PPy onto one side of a 5 mm diameter gold electrode of a 9 MHz AT-cut quartz crystal (8 mm diameter) through a galvanostatic electropolymerization of pyrrole monomer in a buffered solution containing caffeine at room temperature (27 °C).

The electrochemical polymerization set-up was composed of the gold electrode of a quartz crystal as anode and a carbon rod as cathode. Only one of the gold electrodes in the quartz was involved in the cell circuit. A current limiting device (National Semiconductor IC LM 334) was connected in series with the cell and with a voltage source (<30 V). A current density of 4 mA/cm^2 was passed through the cell containing 5 mL of a buffered solution containing the monomer (200 mM) and the caffeine template (20 mM) for about 5 min. At the end of the polymerization period, the template caffeine molecule was removed from the polymer matrix deposited on the surface of the quartz crystal by extraction through washing with water.

A non-imprinted polymer was synthesized using the same procedure but without the incorporation of the caffeine template.

2.3. Sensor instrumentation

The oscillation frequency of the piezoelectric quartz crystal was determined by means of an instrumentation system [19] that consisted of a dc power source (Loadstar P50303) connected to a digital frequency counter (Leader LDC–824), an assembled Pierce oscillator circuit based on CMOS device (CD74HCT04E), and a Teflon flow cell. Only the coated electrode of the quartz crystal inside the flow cell is exposed to the analyte solution. Sample solutions were introduced to the flow cell using a peristaltic pump (Ismatec IPC).

2.4. Caffeine measurement

The measurement procedure was done using a stopped-flow mode. The buffer solution was initially allowed to pass through the flow cell at a constant flow rate of 1.2 mL min^{-1}. The resonant frequency was monitored until it reached a steady-state reading (F_a). Then, solutions of different concentrations of caffeine were successively passed through the cell. For each solution, the flow was stopped and the frequency was recorded till it displayed a constant value (F_b). The sensor response was obtained from the frequency shift ($F_a - F_b$) for each concentration. The same measurement procedure was carried out using same condition without the template.

2.5. Surface analysis of the imprinted PPy film

The imprinted PPy films were characterized using microscopy and spectroscopic surface analysis techniques. The electro-deposited polymer films on the gold electrodes were used as samples for scanning electron microscopy (SEM) and X-ray photoelectron spectroscopy (XPS) analyses.

The surface morphology of the coated quartz was characterized using a JEOL JSM-5200 Scanning Microscope, operated at a voltage of 15 kV, a current of 10^{-12} to 10^{-9} A and a magnification of 3500×. The samples were gold sputtered at 15 kV for 200 s using JEOL JFC-1100E ion sputter (fine coat) operated under vacuum ($\sim 10^{-3}$ Torr).

A Kratos AXIS HSI in an ultra high vacuum (9×10^{-9} Torr) with an Al Kα X-ray source (1486.6 eV of photons) was employed for the XPS analysis of the surface for both the molecularly imprinted polypyrrole (MIPPy) and non-molecularly imprinted PPy coating. To compensate for the surface charging effects, all binding energies were referenced to the C 1s neutral carbon peak at 284.6 eV. The software package XPS PEAK 4.1 was used to fit the XPS spectra peaks.

3. Results and discussion

3.1. Electrodeposition of molecularly imprinted polypyrrole

Electrooxidation of the pyrrole monomer occurs at the anode, and the resulting polymer deposits onto the surface of the gold electrode in the quartz crystal. During the electrodeposition of the conductive polymer, caffeine template molecules are trapped in the polymer matrix as a result of the ability of these molecules to interact with the pyrrole units.

The oxygen atom in the C=O group of the caffeine molecule forms a hydrogen bond with the hydrogen atom in the N–H group of the pyrrole units (Fig. 1). Hydrogen bonding could also occur between the imine nitrogen atom in the caffeine structure and the hydrogen atom of the N–H group of pyrrole. Chain branching and crosslinking in PPy [20] generate a three-dimensional matrix with niches containing the template caffeine. Subsequent washing with water extracts the entrapped caffeine that leaves behind vacant imprint sites bearing its shape. This imprinting process creates a microenvironment for the recognition of caffeine molecule based on shape selection and positioning of the functional groups.

3.2. Effect of electro-polymerization parameters on sensor behavior

The piezoelectric quartz crystal containing the molecularly imprinted polyprrole film constitutes a chemical sensor for caffeine. When the sensor was placed in a solution of caffeine, the oscillation frequency of the quartz crystal decreased significantly, reaching a steady state in about 10 min. The remarkable decrease in frequency indicates an increase in mass on the surface of the quartz crystal, and can be ascribed to the recognition and rebinding of caffeine to the imprinted polymer coating. The lowering in oscillation frequency upon exposure to a solution of caffeine is taken as the sensor response. This behavior was not exhibited by a quartz crystal containing the non-molecularly imprinted polypyrrole, wherein a minimal and erratic response was obtained.

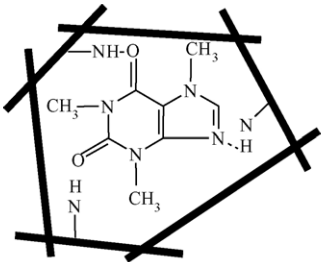

Fig. 1. Interaction between the caffeine molecule with polypyrrole.

The sensor behavior was influenced by the conditions employed in the electrodeposition process. The polymerization parameters such as current density, polymerization time and monomer concentration, determine the thickness and physical stability of the polymer film produced on the sensing surface of the piezoelectric quartz transducer and are expected to have an effect on the sensor response.

The current density applied to the cell during polymerization was found to affect the sensitivity and linearity of the sensor. Sensors produced at lower current densities exhibited favorable analytical performance, whereas the sensors formed at a high current density (e.g. 6 mA/cm^2) showed very low linearity and sensitivity (Table 1). Higher current densities lead to a more extensive electropolymerization, and therefore to the formation of thicker sensing membranes with less accessible imprinted sites. A current density of 4 mA/cm^2 produced a sensor with a high sensitivity (255 Hz/ln concentration) and good linear behavior ($r = 0.9882$), and was therefore used in the subsequent electro-polymerization processes.

The length of the polymerization time was observed to have an influence on the performance of the sensor. A short reaction period (e.g. 1 min) yielded a sensor with small responses, very low sensitivity and poor linearity. Under this condition, not enough electropolymerization must have occurred, producing a thin sensing membrane with only a small number of recognition sites. However, increasing the polymerization time up to 10 min led to an improvement of sensor behavior, possibly due to the formation of more recognition sites. The optimum polymerization time was found to be 5 min, wherein the resulting sensor possessed a high sensitivity (320 Hz/ln concentration) and very good linear behavior ($r = 0.993$).

The monomer concentration during polymerization also determined the analytical behavior of the sensor. Electropolymerization using low monomer concentrations (up to 200 mM) produced sensors with good analytical behavior. Increasing the monomer concentration to 300 mM led to higher sensor responses and increased sensor sensitivity, but caused a highly significant narrowing of the dynamic range. This effect could be attributed the production of a membrane with too many recognition sites that causes overloading of the quartz resonator crystal even at a low analyte concentration (i.e. ca. 0.8 mg/mL). The optimum behavior was observed at a monomer concentration of 200 mM in the presence of 20 mM caffeine and polymerization time of 5 min using 4 mA/cm^2.

Table 1
Analytical performance characteristics of caffeine sensors produced at different current densities

Current density (mA/cm^2)	Correlation coeffient, r	Sensitivity (Hz/ln concentration)
2.0	0.9953	213
4.0	0.9882	255
6.0	0.6625	32

3.3. *Sensor performance characteristics*

The sensor response was very reproducible, exhibiting a relative standard deviation of 9% ($n = 6$) for a solution containing 0.5 mg/mL caffeine. Regeneration of the sensor was done by allowing a water–acetic acid (1:10) mixture to flow in the sensor system for about 2–10 min in order to extract the bound caffeine. Measurement of the buffer solution was done after each regeneration until the frequency reading approaches the initial reading of the buffer solution.

The responses of the caffeine sensor and of the reference sensor to different concentrations of caffeine are shown in Fig. 2. There is a linear relationship between the response, expressed as frequency shift, and the logarithm of the concentration of caffeine in the range of 0.1–10 mg/mL. A good sensitivity (255 Hz/ln mg/mL) was observed for the caffeine sensor. The linearity of the response was good, as indicated by the correlation coefficient ($r = 0.9882$). The detection limit was determined to be 4.66×10^{-6} mg/mL (0.024 μM) based on three standard deviations.

The analytical sensitivity of the caffeine sensor was significantly greater than that of the reference sensor, which had a non-imprinted PPy coating on its surface (Fig. 2). The minimal response exhibited by the reference sensor could be due to the random arrangement of the functional groups in the non-imprinted polymer, resulting in weak and non-specific adsorption.

The response of the sensor varied with the pH of the analyte solution (Fig. 3). The maximum response was obtained at pH 7. This behavior would imply that molecular interaction of caffeine was optimum at this pH, which was also the pH used during the imprinting electro-polymerization process. When basic buffer solutions were used, the response was observed to decrease drastically, indicating that the MIP did not recognize the analyte molecule. Under this condition, the MIP could exist in a highly deprotonated form that is not capable of interacting with the caffeine molecule.

The sensor response was affected by the presence of molecules with structures related to caffeine, particularly theophylline and xanthine. In the presence of a fixed concentration these compounds (0.1 mg/mL), the sensitivity of the sensor to caffeine decreased by about 47% for theophylline and about 68% for xanthine (see Table 2). These results imply that these molecules compete with the caffeine molecule in binding with the MIP. These molecules are also capable of binding with the MIP, since they possess the same functional groups as those that are involved in the interaction between caffeine and the MIP. These molecules differ from the caffeine molecule in having less number of methyl groups and therefore occupy a smaller volume, enabling them to fit into the pores of the MIP. Furthermore, the mass of these molecules are lower than that of caffeine and will therefore bring about a lowering in the sensor response.

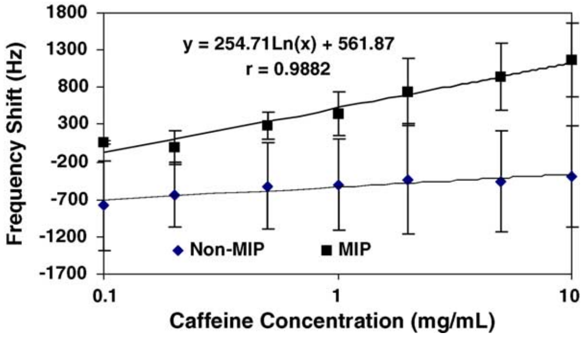

Fig. 2. Response curves obtained using the caffeine (MIP) sensor and reference (non-MIP) sensor.

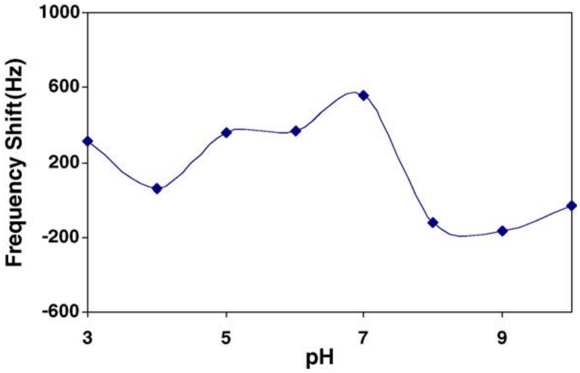

Fig. 3. Effect of pH of buffer solution on sensor response.

3.4. *Surface characteristics of the PPy films*

Scanning electron microscopy conducted at high (3500×) magnification revealed clusters of spherical particles of varying sizes (<5 μm, diameter) on the sensing surface of the MIP sensor. Upon exposure to a solution containing caffeine, the surface appeared to have become slightly thicker, probably due to the incorporation of caffeine molecules in the pores of the polymer matrix (Fig. 4). The surface of the MIP sensor showed more rugged features when compared to the non-imprinted PPy sensor even when exposed to caffeine solution.

The wide-scan X-ray photoelectron spectra of the MIP surface (Fig. 5) indicated the presence of carbon, nitrogen and oxygen. The unexpected presence of oxygen could be attributed to the C–O and C=O groups that could have resulted from the oxidation of some pyrrole rings during the electro-polymerization process [21].

Table 2
Sensor response to various analytes

Analyte	Sensitivity	*r*
Caffeine	255	0.9882
Caffeine with theophylline	140	0.9327
Caffeine with xanthine	84	0.9747

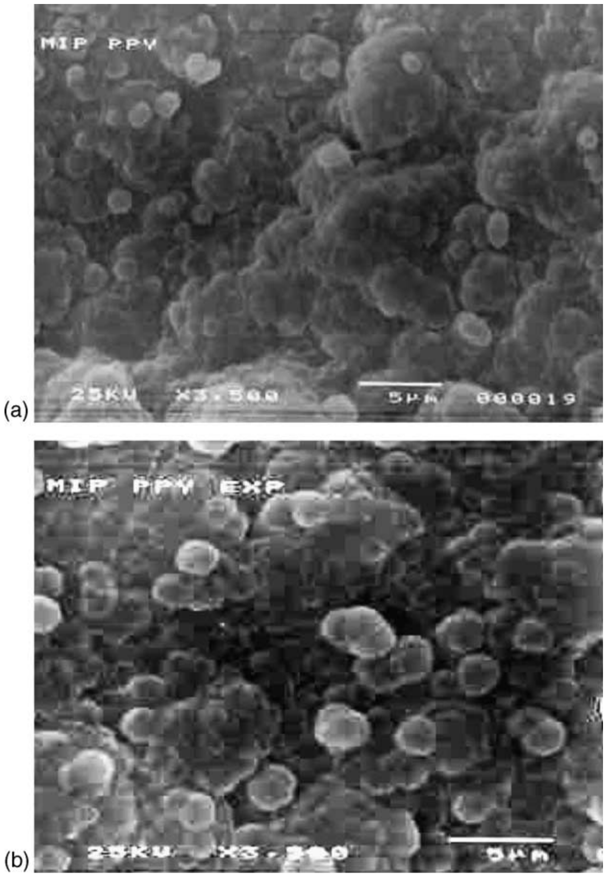

Fig. 4. SEM micrographs of the molecularly imprinted PPy coating at 3500× magnification (a) before and (b) after exposure to a caffeine solution (0.10 mg/mL).

The curve fitted C 1s core level spectrum of the unexposed molecularly imprinted PPy film exhibited three component peaks (Fig. 6(a)). The main peak component is due to a C–C group, which could be a contribution from the aromatic carbons of the PPy. The minor components could be attributed to C–N (286.3 eV) and C=O (288.0 eV) groupings occurring in the polymer film. The possibility of oxidation of the carbons of polypyrrole during the electro-polymerization process could produce a C–OH group in the first step and a C=O grouping in the next step [22]. The high potential (3.7–4.0 V) observed during galvanostatic electropolymerization could facilitate this oxidation.

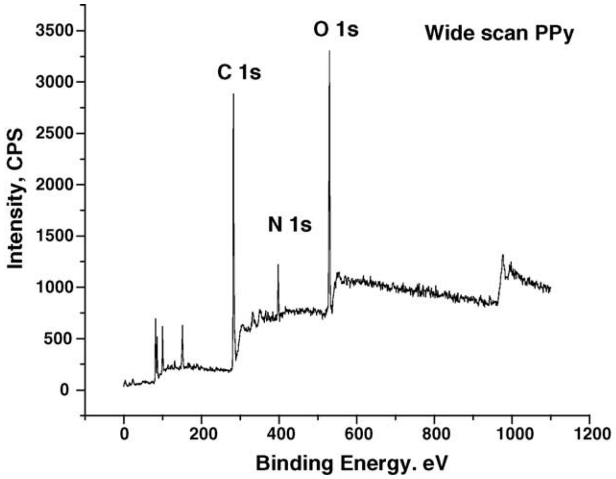

Fig. 5. XPS wide scan spectra of molecularly imprinted PPy coating.

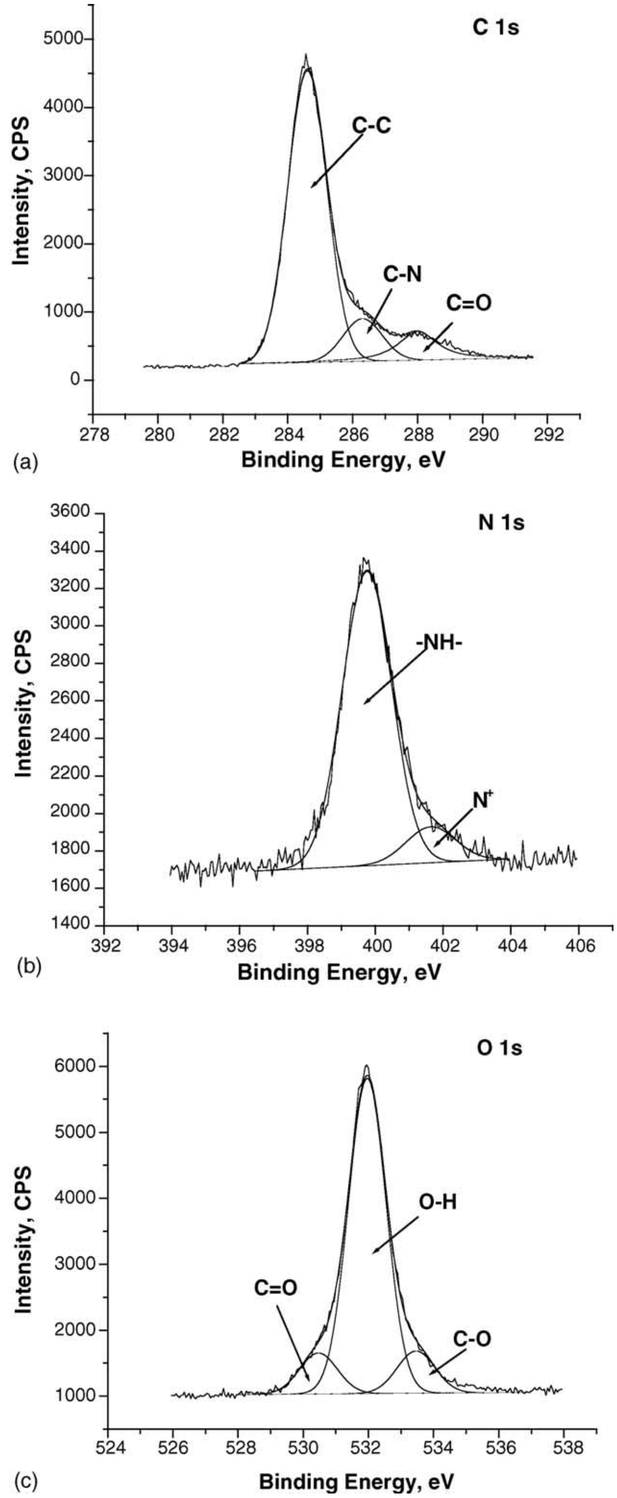

Fig. 6. Curve-fitted XPS spectra of (a) C 1s, (b) N 1s and (c) O 1s components of the molecularly imprinted PPy.

In the PPy complex, the N atoms may exist as imine (=N–), amine (–NH–) and/or electron deficient nitrogen (N$^+$) [11]. The curve fitted spectrum of N 1s (Fig. 6(b)) showed the presence of two types of nitrogen: an amine-like nitrogen (BE $\sim$ 399.5 eV) and electron deficient nitrogen (BE $\sim$ 401.5 eV). The proportion of the positively charged nitrogen atoms in the PPy film was found to be 12% in terms of N$^+$/N. The absence of imine-like nitrogen atom is apparent since no peak with binding energy lower than 399.5 eV was observed.

The deconvoluted O 1s core level spectrum of the PPy film (Fig. 6(c)) was fitted with three peak components ascribed to O–H ($\sim$531.6 eV), C=O ($\sim$530.5 eV) [23] and C–O ($\sim$533.4 eV) corresponding to the major peak and minor peaks, respectively. The occurrences of these groups confirm the possibility of over-oxidation of the polypyrrole during electropolymerization [21,22].

Comparison of XPS results for the PPy films before and after exposure to caffeine provided evidence for the binding of caffeine to the polymer. The curve fitted C 1s, N 1s and O 1s core level spectra after exposure of the molecularly imprinted PPy film to caffeine solution are shown in Fig. 7(a)–(c). The component peaks fitted in the C 1s core level spectrum (Fig. 7(a)) agree with those obtained for the unexposed PPy film except for the appearance of an additional peak that can be attributed to the C(=O)–N peak ($\sim$287.3 eV). The C(=O)–N component peak is also observed in the C 1s core level spectrum of pure caffeine. Its presence in the XPS spectra of the PPy film exposed to caffeine could indicate that caffeine molecules are present and have been recognized by the PPy sensing layer. The increase in the atomic percentage of the oxygen corresponding to C=O peak upon exposure to caffeine could also be an indication of caffeine recognition on the surface of the polymer matrix.

The showing up of the imine-like nitrogen atom in the N 1s curve fitted peak was obvious in the PPy film upon exposure to caffeine (Fig. 7(b)). This response could also provide an indication of caffeine rebinding to the polymer. In this core level spectrum, the imine-like nitrogen and the electron deficient nitrogen constitute about 9 and 11%, respectively.

For the non-imprinted PPy films, the XPS wide scan spectra revealed peaks for carbon, oxygen and nitrogen. The curve fitted C 1s core level spectra of the non-imprinted imprinted PPy films before and after exposure to caffeine solution (Fig. 8(a) and (b)) both contained three component peaks. Unlike in the molecularly imprinted PPy, no peak was found corresponding to the C(=O)–N grouping, indicating that caffeine was not incorporated in the NIP after exposure to a solution containing caffeine.

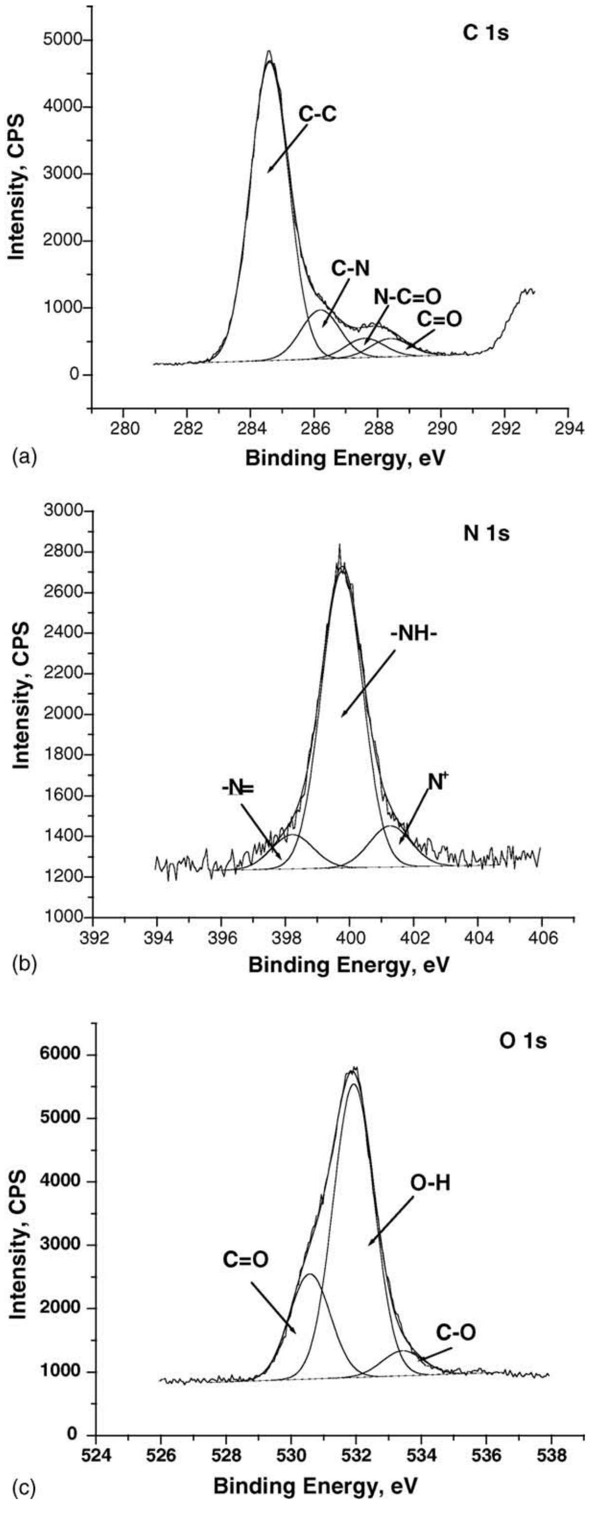

Fig. 7. Curve-fitted XPS spectra of (a) C 1s (b) N 1s and (c) O 1s components of the molecularly imprinted PPy after exposure to caffeine.

The deconvoluted O 1s core level spectrum of the non-imprinted PPy films before exposure to caffeine (Fig. 8(c) exhibited three component peaks, with C=O ($\sim$530.0 eV) as the major peak and the O–H ($\sim$531.6 eV) and COO ($\sim$528.5 eV) as minor peaks. As in the MIP, these groups could be the outcome of over-oxidation of polypyrrole during electropolymerization. Upon exposure to caffeine (Fig. 8(d)) the O 1s spectrum was fitted with two peak components ascribed to C=O ($\sim$529.5 eV) and O–H ($\sim$531.2 eV) as the major and minor peaks, respectively.

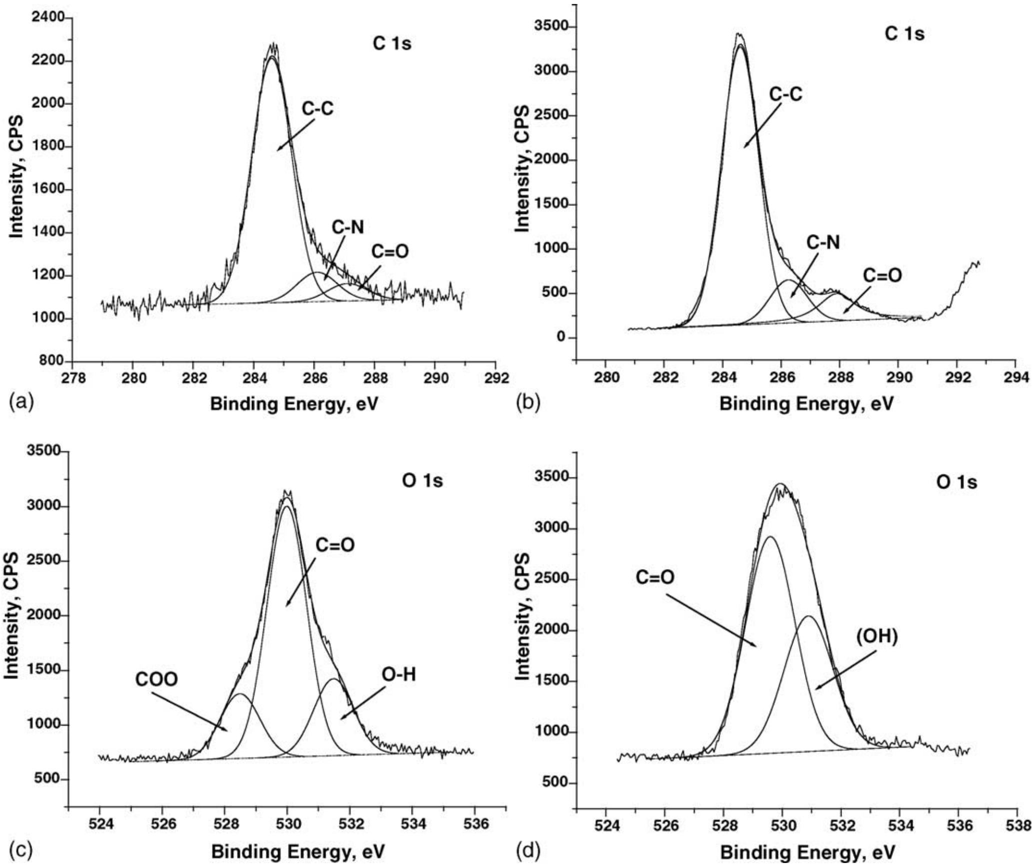

Fig. 8. Deconvoluted spectra of the elemental compositon of C 1s and O 1s of the non-imprinted PPY coating: (a) and (c) unexposed; (b) and (d) exposed to caffeine solution.

The N 1s core level spectrum of the unexposed non-imprinted PPy (Fig. 9(a)) was fitted with a low BE shoulder at 397.6 eV (imine-like nitrogen) as the main peak and a minor component assigned to the amine-like nitrogen atom (∼399.1 eV). Unlike in the molecularly imprinted PPy film, a peak that can be attributed to a protonated nitrogen was not found. This could suggest that the presence of the template caffeine during the polymer synthesis of the film affected the surface structure of the PPy, wherein the PPy film assumed a deprotonated form [22] after its extraction. On the other hand, PPy synthesis without the caffeine resulted in a protonated form [22] containing a positively charge nitrogen N^+ and a neutral nitrogen (–NH–).

Very minimal difference was observed between the non-imprinted PPy before and after exposure to caffeine solution, suggesting the non-binding of caffeine. After exposure to caffeine, the curve fitted peak for the N 1s core level spectrum (Fig. 9(b)) revealed a major peak for the amine-like nitrogen atom (399.4 eV) and minor peaks attributed to the protonated nitrogen atom (N^+) and the imine-like nitrogen atom. This behavior was also observed for the N 1s of the molecularly imprinted PPy after exposure to caffeine. With these observations, very weak interaction of caffeine on the non-imprinted polymer could also be manifested on the non-imprinted PPy film.

3.5. *Analytical application of the sensor*

The suitability of the sensor for analytical applications was demonstrated by the recovery efficiency in analyzing coffee samples. Percent recovery for brewed coffee (4 g/50 mL) ranges from 74 to 93%. The results of the analysis using the sensor were compared with those obtained using the standard spectrophotometric method, AOAC Method 12.028, [24] involving chloroform extraction and subsequent absorbance measurement on the extract at 276.5 nm wavelength (see Table 3). A *t*-test (95% confidence level) performed on these data showed that there was no significant difference between the results obtained with the two methods.

The sensor was found to be useful for only three measurements, after which its performance deteriorated. This limited lifetime could be an effect of the acidic nature of the re-

Table 3
Estimated caffeine content of the coffee sample (4 g/50 mL) using the caffeine sensor with molecularly imprinted PPy coating and UV–vis spectrophotometer

Method	Caffeine (mg/mL)	S.D.	*n*
MI-PPy sensor	0.988	0.198	3
UV–vis	1.103	0.047	3

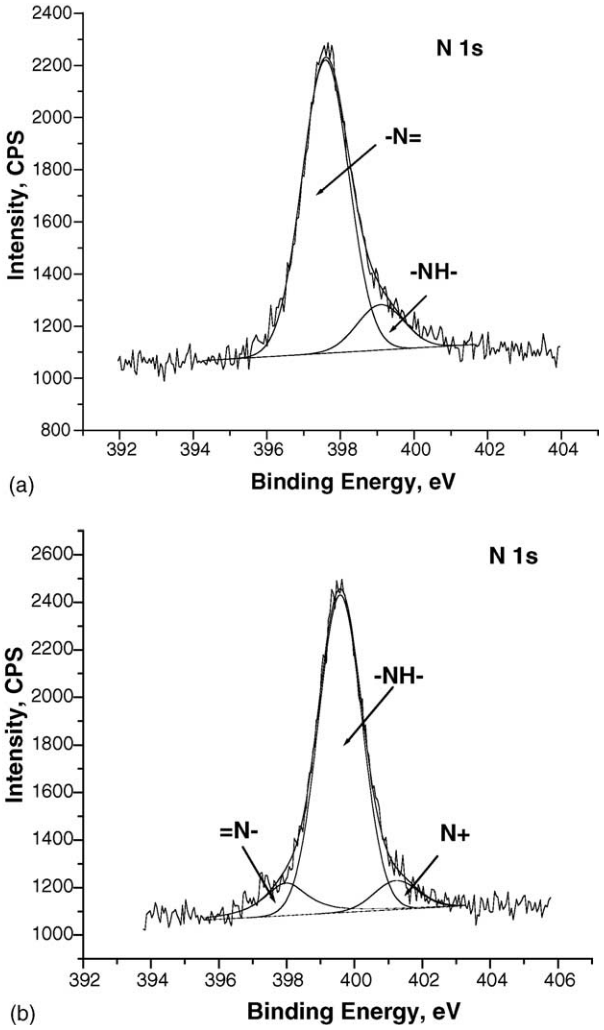

Fig. 9. Deconvoluted spectrum of the N 1s elemental compositon of (a) unexposed non-imprinted PPY coating and (b) non-imprinted PPy coating exposed to caffeine solution.

generating mixture, which could cause modification in the recognition site, and even in the polymeric backbone.

4. Conclusion

The approach to preparing a chemical sensor through the combination of electrosynthesized molecularly imprinted polypyrrole and the piezoelectric quartz sensor has been successfully demonstrated. This promising sensor exhibited a linear calibration range, a good sensitivity and repeatability. Molecular imprinting by electropolymerization of PPy was shown to be an effective way of growing the polymer coating directly onto an electrode of the quartz crystal using galvanostatic method. The proposed low cost chemical sensor could find application in the measurement of caffeine level in food and pharmaceutical industries.

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