

Research Article

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Preparation and characterization of cellulose paper/polypyrrole/bromophenol blue composites for disposable optical sensors

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Abstract: This manuscript describes a novel approach for the preparation of composites based on cellulose paper (CP) modified with polypyrrole (PPy) and bromophenol blue (BPB) (CP/PPy/BPB). Cellulose fibers are suitable for uniformly retaining the polymer in its doped form, once BPB can be used as a negatively charged dye. The CP/PPy/BPB composites were characterized by Raman Spectroscopy, Scanning Electron Microscopy, Spectroscopy and Dispersive Energy, and X-ray Diffraction. After characterization, they were studied for the quantification of pH and ammonia by diffuse reflectance spectroscopy. Under varying pH, different colors were verified, which ranged from yellow to blue, and an optical response for ammonia at concentrations as high as 25 ppm. The replacement of BPB by bromothymol blue (BTB) did not produce efficient color transitions and showed no sensitivity to changes in pH and ammonia concentration.

Keywords: Cellulose, composites, polypyrrole, dyes, optical sensors.

1 Introduction

Cellulose has been used as a matrix for the development of composites due to its favorable characteristics, such as availability, biodegradability, low cost, and solvent-absorbency [1, 2]. More recently, special emphasis has been given to the use of cellulose paper (CP) in miniaturized and disposable sensors of different shapes and sizes [3, 4]. Among the matrices used in sensors, CP has been efficiently

chosen for detecting colorimetric changes at different pHs and ion concentrations (Cu and Fe) [5]. In this experiment, CP immersed in a solution containing a dye was studied for the capacitive detection of changes in sweat, which contains primary ions, lactate and ammonia [5]. The choice of a particular method for optical sensing usually depends on the development of interdigitated microelectrodes by several preparation steps. Here, we propose a simple methodology for the preparation of CP-based composites. In opposition to more complex layered structures found in the literature, this methodology reduces production steps and costs. The use of bromophenol blue (BPB) as a sensitizing dye was investigated after the incorporation of a stable conducting polymer (polypyrrole, PPy) onto a CP matrix.

In situ chemical polymerization is a useful methodology for the preparation of composites based on conducting polymers [6, 7]. In this case, PPy is a suitable option due to its relatively high conductivity, stability in air, and biocompatibility [8, 9]. Previous results have reported an efficient deposition of PPy films on solid electrodes (Au and ITO) by electrochemical oxidation of pyrrole in the presence of a sensitizing dye, i.e. bromophenol blue (BPB), which behaves as a doping agent for PPy [10–12]. For simplifications in the experimental methodology, composites were prepared here by *in situ* polymerization of pyrrole on a flexible CP matrix modified with BPB, and fabricated in different sizes and shapes. Such a system precludes the viability of chemically preparing CP-based sensors by the immobilization of an acid-basic indicator (BPB) for the quantification of solutions within a reliable pH range.

Indicator dyes have been immobilized, embedded or encapsulated in different matrices, such as gelatin, polymer, and sol-gel matrices [10–15]. Other studies have focused on the preparation of PPy- α -cellulose composites for the removal of red dye from aqueous solutions [16]. The present study proposes a mechanism of dye incorporation based on diffusion and adsorption

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within thin PPy shells [16]. PPy, among other conducting polymers, are of interest for sensing applications due to a strong pH dependence after varying their oxidation state (*p*-doping and *n*-doping). The generation of charges into a conducting polymer combine electrical conductivity with optical properties, and when in the presence of a dye, it allows one to obtain large changes in their optical spectra on exposure, for instance, to gases, humidity, and acid-base solutions [17, 18].

The use of a versatile cellulose matrix, as CP modified by a conducting polymer, seems an appropriate strategy for the development of accurate optical sensors after the incorporation of a dye. However, strong absorbing conducting polymers deposited onto opaque CP matrices are hardly compatible with spectroscopic detection. Therefore, the use of diffuse reflectance spectroscopy (DRS) is proposed for the characterization of CP/PPy/BPB of varying sizes and shapes.

Although commercial pH sensors are available, there is a great interest in the fabrication of disposable sensing systems, in particular if they are inexpensive, simple, and reliable for general proposes such as epidermal sensing. When they are compared to sensors build on rigid electrodes, paper-based sensors have advantages such as better uniformity and a strong impregnation with other components yielding good candidates for composite materials applied on paper devices and sensors.

2 Experimental

2.1 Materials

Pyrrole (monomer, 98%, Sigma-Aldrich) was purified under reduced pressure and stored in a fridge under light protection. The oxidant (copper chloride, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), BPB and bromothymol blue (BTB) were used as received (Sigma-Aldrich). CP (GB003 grade blotting paper, Whatman, Pittsburgh, PA, USA) was cut into sheets of different sizes, for general experiments, and as 0.8-cm diameter disks, for diffuse reflectance measurements. Analytical grade isopropanol and standard buffer solutions were obtained from Fisher Scientific (Pittsburgh, PA, USA).

2.2 Sample preparation

The CP matrices were previously treated in a solution containing BPB (eventually BTB) in isopropanol at 0.5 mg mL^{-1} under stirring for 1 h followed by drying with N_2 stream.

In a typical procedure for the *in situ* polymerization of pyrrole, BPB was initially dissolved in isopropanol at 0.5 mg mL^{-1} . Pieces of previously treated CP were added to this solution under stirring. Two solutions were then prepared separately in isopropanol, an oxidant solution at 0.05 mol L^{-1} and a monomer solution at 0.2 mol L^{-1} . The monomer solution was added drop by drop under stirring to the oxidant solution containing immersed pieces of CP. The polymerization time was defined as 30 min because it yielded a uniform coverage of PPy onto CP. The composites obtained (CP/PPy/BPB) were kept in isopropanol for 10 min under stirring for the elimination of powdery materials eventually deposited on the CP surface. For comparison purposes, CP/PPy (without BPB) was also prepared by a similar procedure. All samples were dried in a ventilation hood at room temperature for 2 h.

2.3 Characterization

The composites were analyzed by micro-Raman spectroscopy (Nanophoton Laser Raman microscope, Raman-11) with a high power laser (785 nm) and acquisition time of at least 10 s/shot. The use of a single-frequency wavelength-stabilized laser microscope enabled the selection of specific morphological features of cellulose fibers.

A JEOL JSM-6060 LV Scanning Electron Microscope (SEM) was used for the morphological analysis and a chemical analysis was performed by energy dispersive X-ray spectroscopy (EDS) (Oxford Instruments ISIS EDS System).

The samples were also characterized by a Philips X-Pert diffractometer, with a $\text{Cu-K}\alpha$ target at $\lambda = 1.5418 \text{ \AA}$. Data were collected in a step-scan mode from 5° to 55° at 2° min^{-1} , 10 mA and an accelerating voltage of 45 kV. XDR simulations were conducted on MDI Jade software.

A double-beam double-grating spectrometer (Cary UV-VIS-NIR 5G) was used for diffuse reflectance measurements. A pressed polytetrafluoroethylene (PTFE) disk was used as a reflectance standard and all spectra relative to the PTFE disk were recorded (relative reflectance, %R). The wavelengths ranged from 350 nm to 800 nm and evidenced changes in the spectra and no corrections in intensity were required. The CP matrix is not transparent under the wavelength range studied and its spectrum showed a large signal with no well-defined band. Such a signal was used for the subtraction of the background. The background spectra were obtained for CP at conditions similar to those used for CP/PPy/BPB and CP/PPy. Net signals were calculated for each measurement

by the subtraction of background signals from calibration curves, i.e., for CP/PPy/BPB, its spectra were obtained before and after the testing response in buffer solutions at different pHs. The final spectra were obtained after the subtraction of the signals from the original matrix (CP or CP/BPB).

3 Results and discussion

3.1 Preparation procedures

The procedures for the synthesis of PPy when modified with BPB and then, deposited on CP were optimized. The following conditions were tested: *i*) polymerization media (water, acetonitrile or isopropanol), *ii*) reaction time (from 30 min to 5 h), *iii*) concentrations of each component (pyrrole and BPB), *iv*) immersion time of CP into the polymerization medium at different reaction stages, *v*) filtration of the polymerization solution at different stages for the avoidance of powdered materials deposited on the CP surfaces, and *vi*) immersion of CP into the reaction medium after its treatment in BPB dissolved in buffer solutions at two pH values (2.0 and 10) and two periods of time (1 h and overnight).

After the experiments, the following protocol was defined as the best: isopropanol as the solvent for the preparation of all solutions, monomer (pyrrole) in excess (at 0.2 mol L^{-1}), oxidant at 0.05 mol L^{-1} , previous immersion of CP in a BPB solution at 0.1 mg mL^{-1} for 1 h prior to the reaction, and immersion of the CP/BPB matrices into the reaction medium over the whole reaction time (30 min). Isopropanol was chosen as the solvent, since it yielded stable colors for the CP matrix after modification with PPy and BPB.

3.2 Structure characterization

The Raman spectra identified chemical differences in the CP matrix before and after modification with PPy and BPB (Figure 1). The Raman spectrum of CP showed the main band of crystalline cellulose at 1485 cm^{-1} with a very small contribution of amorphous cellulose due to the absence of a well-defined band at 1462 cm^{-1} (not seen in the spectra). Such two bands have been used for the estimation of crystallinity for cellulosic materials [19]. Other important bands, at 1100 cm^{-1} and 1126 cm^{-1} , can be assigned to symmetric C-O-C stretching, and those at 1158 cm^{-1} to asymmetric C-C and C-O stretching of cellulose [19]. Two other bands, at 1348 cm^{-1} and 1383 cm^{-1} , can be assigned

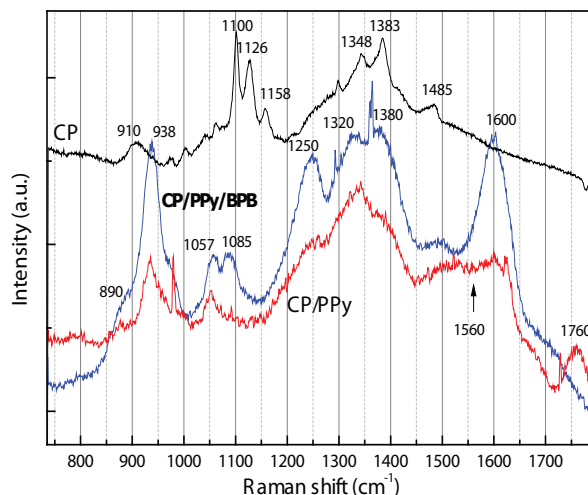


Figure 1: Resonance Raman spectra for CP, CP/PPy and CP/PPy/BPB.

to the bending of HCH, HCC, HOC and COH groups of cellulose.

The Raman spectra of CP/PPy and CP/PPy/BPB indicated a doping process of PPy when deposited on the cellulose fibers. The bands at 938 cm^{-1} , 1085 cm^{-1} , 1380 cm^{-1} , and 1600 cm^{-1} for CP/PPy and CP/PPy/BPB, can be assigned to an oxidized PPy structure [20, 21]. Such signals are well-defined in the spectrum of CP/PPy/BPB, which indicates BPB as a doping agent for PPy. This particular effect resulted in the band at 1600 cm^{-1} assigned to aromatic C-C and C=C stretching of doped PPy [20, 21]. On the other hand, the spectrum for CP/PPy (without BPB) shows a large band at 1560 cm^{-1} due to PPy in its neutral, non-doped state [20].

The band at 1600 cm^{-1} , typically related to a doped PPy structure, elucidated differences in distribution in conjugation lengths of PPy [20]. In this case, both intensity and definition at 1600 cm^{-1} are remarkably different for CP/PPy and CP/PPy/BPB. Moreover, an additional band, at 1760 cm^{-1} , is defined only for CP/PPy, and can be assigned to C=O groups present in the structure of PPy due to some degree of overoxidation.

The Raman spectrum of CP/PPy/BPB also shows a band at 1050 cm^{-1} , which has merged to two bands, one at 1057 cm^{-1} and another at 1085 cm^{-1} . Both bands can be attributed to in-plane C-H deformation of doped PPy and corroborate the assumption BPB is a doping agent for PPy. Finally, the typical bands of pure BPB are not so evident for CP/PPy/BPB, once they were masked by other strong bands of PPy. However, the bands at 890 cm^{-1} , 1085 cm^{-1} and 1320 cm^{-1} can be related to the presence of BPB and are observed in the spectrum of pure BPB dissolved in buffer solutions [10].

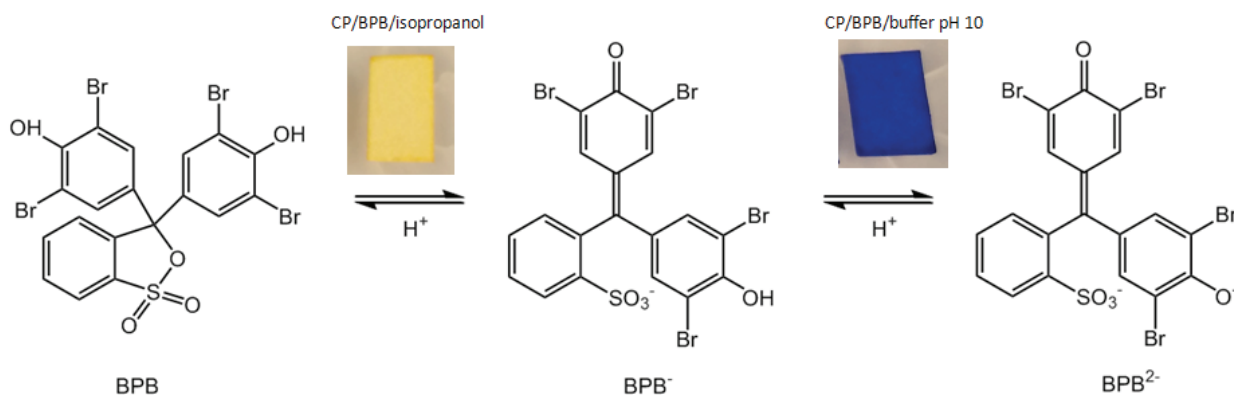


Figure 2: Chemical structures of neutral to negatively charged BPB and images of CP after immersion in BPB in: isopropanol (yellow) and a buffer solution at pH 10 (blue).

3.3 Doping transitions

PPy changes from neutral to doped state in the presence of BPB dissolved in a solvent such as isopropanol. In this case, BPB acts as a doping agent for PPy due to the presence of functional sulfonate groups in its structure. BPB changes from neutral to negatively charged (Figure 2), according to the medium used for its dissolution, i.e., BPB is in its anionic form (BPB⁻) (yellow) at low pHs and in isopropanol solutions and dianionic form (BPB²⁻) (blue) at high pHs.

Figure 2 also shows pictures of CP after immersion in BPB dissolved in isopropanol (yellow) or a buffer solution at pH 10 (blue). After overnight immersion, such uniform colors seen for CP/BPB at different pHs become even more intense. When immersed for longer times in different buffer solutions, such as at pH 2 or pH 10 containing BPB, CP becomes swollen because water enters cellulose fibers and produces an amorphous structure, as verified by X-ray diffraction (Figure 3). This result can be related to the form of BPB, which is anionic (BPB⁻) in isopropanol or in standard buffer solution at pH 2, and dianionic (BPB²⁻) at a standard buffer solution at pH 10.

3.4 X-ray characterization

Differences in crystallinity for CP treated in isopropanol or buffer solutions can be related to the nature of the solvent, which may induce different evaporation rates and penetrate differently within the cellulose fibrils. The diffraction patterns are different for CP/PPy/BPB when prepared in a solution containing BPB in isopropanol or in a standard buffer solution at pH 10. A more crystalline structure is observed for CP/PPy/BPB prepared in isopropanol (Figure 3).

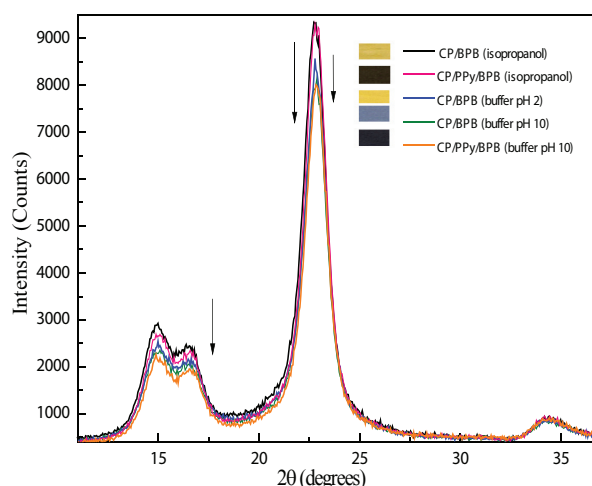
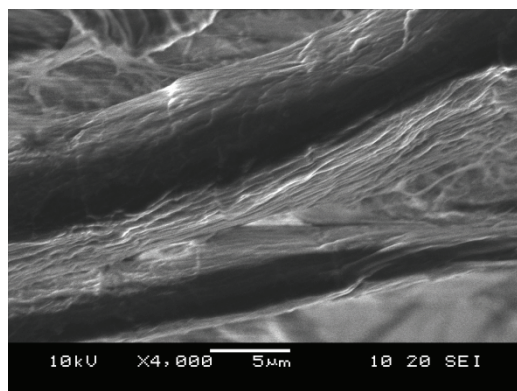
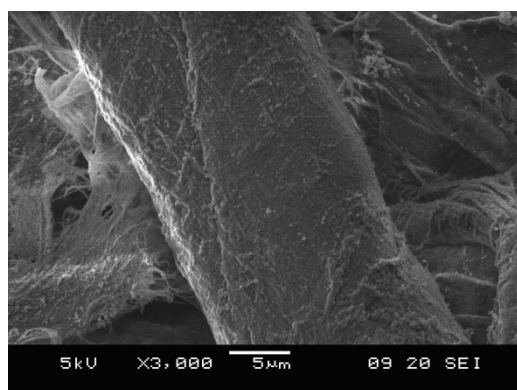
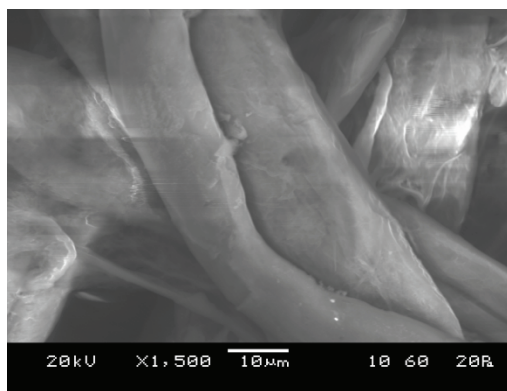


Figure 3: XRD patterns of CP/BPB (prepared in isopropanol and standard buffer solutions at pH 2 and pH 10) and CP/PPy/BPB (prepared in isopropanol and standard buffer solution at pH 10) with images from these materials.

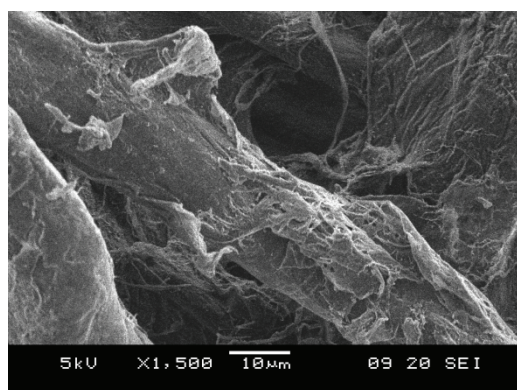
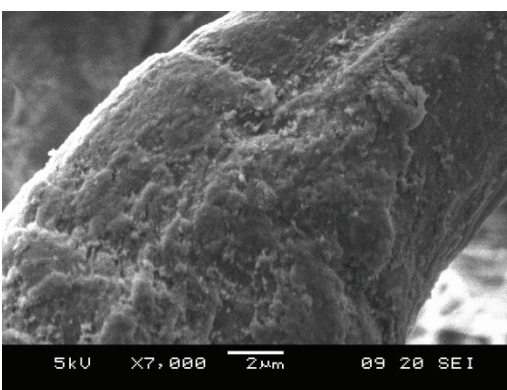
The diffractograms of CP, CP/PPy and CP/PPy/BPB show the typical pattern of cellulose in its crystalline state (cellulose I), i.e., two less intense peaks at 2θ = 14.9 and 16.6 and a sharp peak at 2θ = 22.8 assigned to [110], [110], [002] crystalline planes of native cellulose, respectively [21, 22]. Such peaks are less intense for CP/PPy, since PPy is in its non-doped (amorphous) state. In this case, when the cellulose fibrils are covered by a non-conductive PPy, crystallinity is expected to be lower. Accordingly, the relative crystallinity (X_c) was calculated through the integration of the total area under the diffractograms from 10° to 50° and background (non-crystalline) area. The difference between such values enables the calculation of the area of the crystalline peaks related to the total area, i.e. the value of X_c . The values of X_c were 50.6%, 39.3%,



(a) Cellulosepaper (CP)



(b) CP/PPy composites



(c) CP/PPy/BPB composites

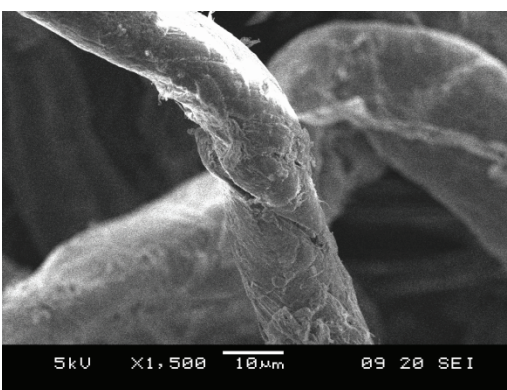


Figure 4: SEM images for: CP (a), CP/PPy (b) and CP/PPy/BPB (c).

and 33.8% for CP, CP/PPy/BPB and CP/PPy, respectively, and indicate PPy had penetrated, covered the cellulose fibers, and yielded a reduced crystallinity.

3.5 Morphology analysis

The morphology of CP was analyzed after modification with PPy and BPB. Figure 4a shows the SEM images of CP with randomly oriented cellulose fibrils on smooth surfaces,

similarly to the images taken for cotton fibers [21]. After modification with PPy (Figure 4b) and PPy/BPB (Figure 4c), the fibers became swollen and uniformly covered. The presence of aggregates is evident for CP/PPy/BPB (Figure 4c) with its rough surface due to the deposition of PPy and BPB over the cellulose fibers.

Figure 5 shows the EDS spectra for CP/PPy and CP/PPy/BPB with typical signals of the cellulosic matrix (C and O atoms) and those of BPB (Br and S atoms). The small signal for Cl can be related to the oxidant used (CuCl_2). The

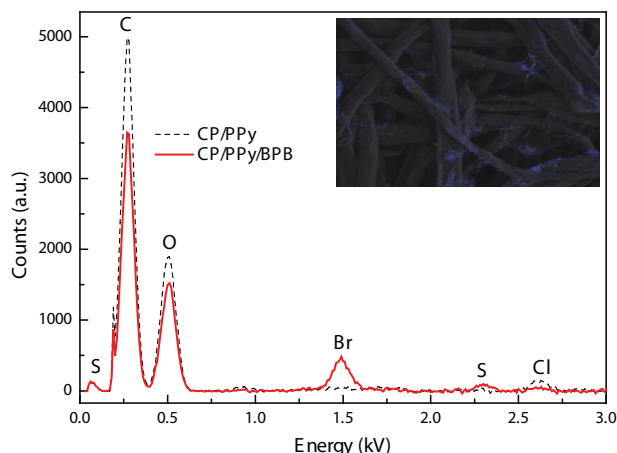


Figure 5: EDS for CP/PPy and CP/PPy/BPB.

insert of Figure 5 shows the SEM image of CP/PPy/BPB with adsorbed Br atoms from BPB over the cellulose fibers.

3.6 Analysis in Diffuse Reflectance Spectrometry

Diffuse Reflectance Spectrometry (DRS) was used as a non-destructive technique for comparative analysis of CP/PPy and CP/PPy/BPB. At least three measurements were performed for the verification of the repeatability over different days and samples. Even when prepared under the same conditions, CP/PPy and CP/PPy/BPB rarely showed the same relative intensity due to small differences in homogeneity and surface loading. This limitation was also verified for CP as related to variations in the packing density and homogeneity (scattering effects) in cellulose. Despite such differences in intensity, which varied from *ca.* 10% to 25%, no change in the band shape was observed in the DRS spectra for the samples prepared under similar conditions over at least three repetitions.

Figure 6 shows the DRS curves obtained for CP/PPy/BPB after immersion in buffer solutions at different pHs. One drop of each buffer solution was added separately to the composites by a syringe (with the same volume, 5 μ L), *i.e.*, several samples received a drop of a specific buffer solution at the same time.

The samples were dried with gentle N_2 stream and the measurements were successively taken for each sample. The background was obtained separately for each sample prior to the dropping of the standard buffer addition and the spectra were obtained 5 min after the buffer solutions had been dropped onto each surface. This methodology enables the comparison of signals for samples with small

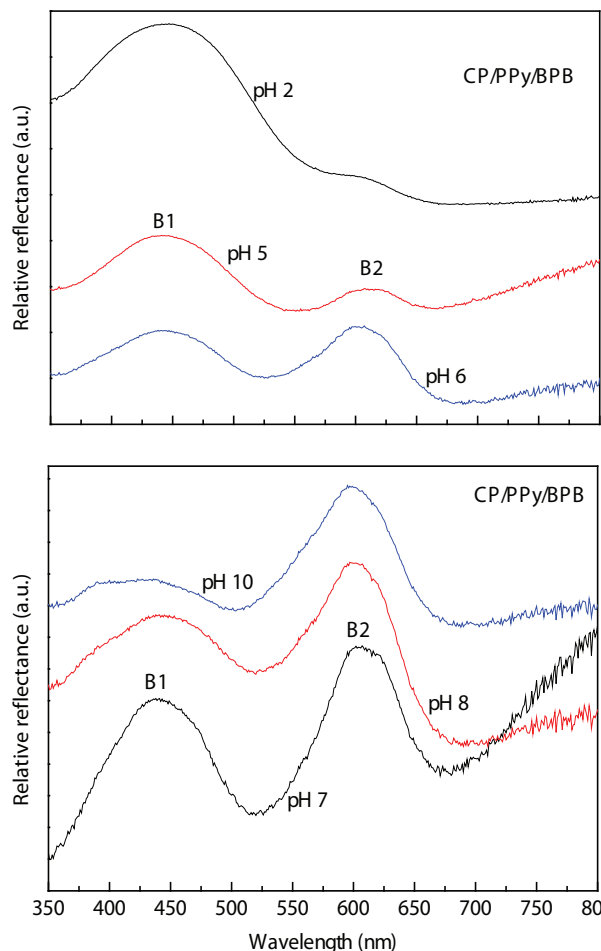


Figure 6: DRS curves for CP/PPy/BPB at different pHs.

changes in tonality, since the background of each sample had been subtracted (net signals).

Although all samples were prepared under the same conditions, small differences might be detected in coverage or color intensity; therefore, by subtracting the background (blank signal), differences in uniformity were attenuated. The DRS signals also showed small differences in intensity with the drying time of the samples. However, for a period of at least 1 h, no significant difference was verified in the signal intensity. The composites became lighter with time (days) due to evaporation of components, but this effect was not systematically studied. It suggests single uses as disposable sensors, which is most commonly seen for cellulose-based materials.

Figure 6 shows two well-defined bands at 450 nm and 600 nm (B1 and B2) for CP/PPy/BPB. In this situation, PPy is in its doped form and BPB is in its anionic form for the CP/PPy/BPB composite. Therefore, the spectrum of CP/PPy/BPB at pH = 2 shows a typical curve with an intense

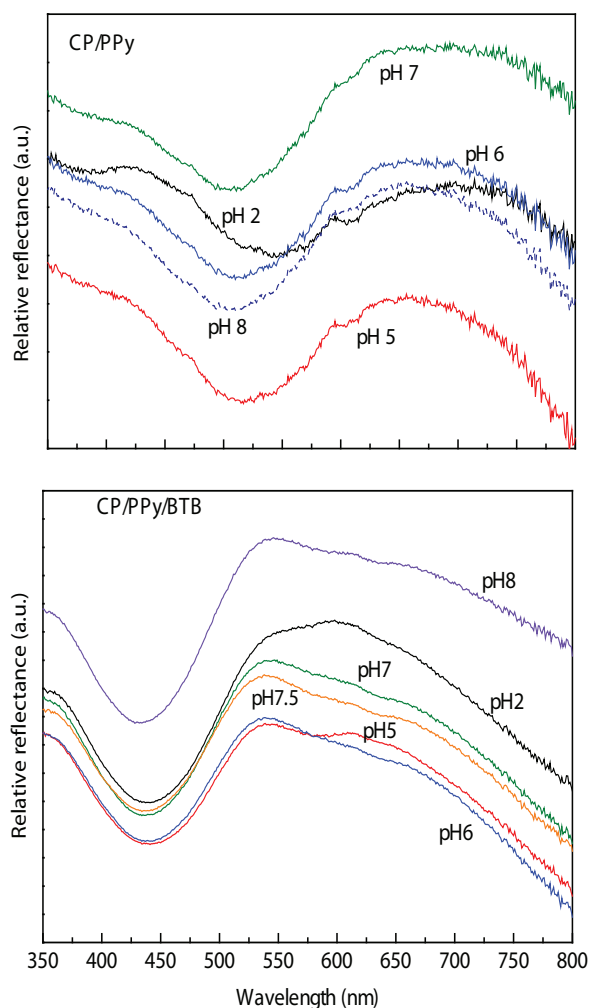


Figure 7: DRS curves for PPy and CP/PPy/BTB at different pHs.

B1 band. When pH is higher, protons are extracted from the BPB structure and a blue form for BPB is expected above pH 6.0.

The bands in Figure 7 for CP/PPy are similar to those of BPB in the solution (results not shown), which indicates the binding of BPB in a cellulosic matrix modified with PPy ensures color transitions in buffer solutions. BPB in solution typically shows yellow, red and blue colors, according to the pH [10, 11]. When originally obtained, CP/PPy was a non-doped form of blue color and its spectra showed no response to variations in pH. A low sensitivity to pH was also verified when bromothymol blue (BTB) was tested (Figure 7). In this case, a blue composite was obtained (CP/PPy/BTB).

The CP/PPy/BPB composite was also tested for ammonia, which is one of the waste products from sweat and essential to many biological reactions [5]. Aliquots of ammonia in an aqueous solution were added to the

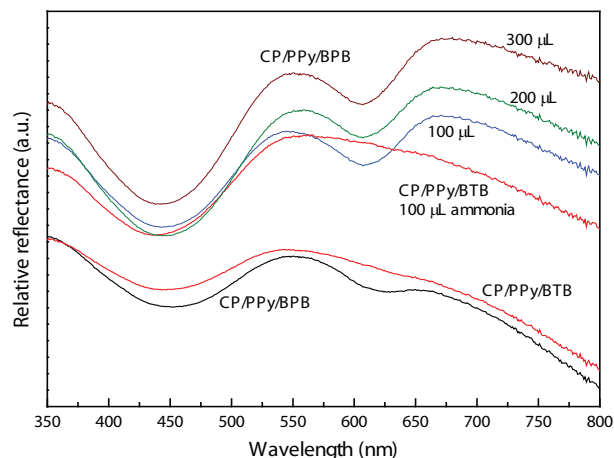


Figure 8: DRS curves for CP/PPy/BPB before and after adding 100 μL (10 ppm), 200 μL (20 ppm) and 300 μL (30 ppm), and CP/PPy/BTB, 100 μL (10 ppm) de ammonia.

sample surfaces (drops of 100 μL from a NH_4Cl stock solution), which resulted in different responses for CP/PPy/BPB (Figure 8), thus evidencing that only CP/PPy/BPB responds to ammonia.

A quantitative analysis of the role of CP/PPy/BPB as a sensor can be conducted by decreasing interference effects and normalizing and studying the effects of different recovery periods of PPy on the acid-base response of CP/PPy/BPB.

4 Conclusions

Disposable colorimetric CP/BPB/PPy sensors for pH and ammonia have been developed by a straightforward method, which was from *in situ* oxidative polymerization of pyrrole on a CP matrix previously modified with BPB. BPB was incorporated over cellulose fibrils covered with uniform PPy layers. A strong binding of BPB within the cellulose matrix was verified by SEM, EDS, and XRD. The Raman spectra of CP/PPy and CP/PPy/BPB indicated the doping of PPy deposited on the cellulose fibers modified with BPB (Figure 1). The presence of sulfonic groups in BPB enabled its retention as a doping agent for PPy over the cellulose fibers. CP/BPB/PPy exhibited a pH-dependent response with different colors, which indicates an effect of permeability of ions within the CP matrix. In comparison to CP/PPy (without BPB) and CP/PPy/BTB (with bromothymol blue), only CP/PPy/BPB showed sensitivity to changes in pH and ammonia concentration; therefore, CP/PPy/BPB is a promising candidate for acid-base and ion selective sensing.

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