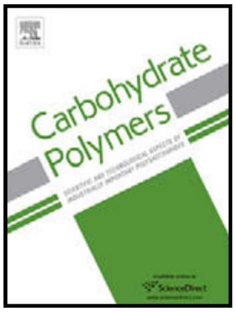

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Optical Devices for the Detection of Cyanide in Water Based on Ethyl(hydroxyethyl)cellulose Functionalized With Perichromic Dyes

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Research Highlights

- Ethyl(hydroxyethyl)cellulose was functionalized with protonated perichromic dyes.
- The systems were designed to be used for chromogenic/fluorogenic anion sensing.
- Films were utilized for the highly selective optical detection of CN^- in water.
- The polymeric chain protects the sensing unit from the medium, improving the detection limit of CN^- in water.
- The films can be reused.

Abstract

Films of three polymers, based on ethyl(hydroxyethyl)cellulose functionalized with protonated perichromic dyes, were used for anion sensing. The polymer functionalized with protonated Brooker's merocyanine acts as a chromogenic/fluorogenic system for the selective detection of cyanide in water. An increase of >28 times was verified for the fluorescence lifetime of the sensing units in the polymer in comparison with protonated Brooker's merocyanine in water. Moreover, an increase in the pK_a values was verified for the sensing units in the polymers. Data suggest that the hydrocarbonic polymeric chains provide an adequate microenvironment to protect the sensing unit from bulk water. The other polymer, functionalized with an iminophenol, also showed high selectivity for cyanide (detection limit = 9.36×10^{-6} mol L^{-1} and quantification limit = 3.12×10^{-5} mol L^{-1}). The polymer functionalized with azophenol units is unable for the detection of cyanide, due to the low pK_a value verified for its chromogenic units.

Keywords: ethyl(hydroxyethyl)cellulose; cyanide; naked−eye detection; chromogenic chemosensors; fluorogenic chemosensors; anion sensing.

1. Introduction

Research on the recognition and detection of anions has increased in recent years due to the very important role played by these species in chemical and biological processes (Anslyn, 2007; Cho & Sessler, 2009; Gai et al., 2015; Gale, 2010; Gunnlaugsson, Glynn, Tocci, Kruger & Pfeffer, 2006; Huynh, Sharma, Sosnowska, D'Souza & Kutner, 2015; Martínez-Máñez & Sancenón, 2003; Suksai & Tuntulani, 2003; Wenzel, Hiscock & Gale, 2012; Yang, Zhao, Feng & Li, 2013; Zimmermann-Dimer & Machado, 2008). An important target analyte in terms of detection is CN^- (Nelson, 2006), which is lethal in very small concentrations because it binds strongly to the active site of cytochrome−oxidase, causing a decrease in the oxidative metabolism (Bhattacharya & Flora, 2015; Luque-Almagro, Moreno-Vivián & Roldán, 2016; Nelson, 2006; Zelder & Männel-Croisé, 2009). CN^- is a chemical species present in several processes, being fundamental in various industrial activities, such as the synthesis of polymers, metallurgy, and mining. In addition, this anion is released in the hydrolysis of cyanohydrins, present in certain fruit seeds and roots, and also of some neurotoxic warfare agents (Agatemor, 2009; Nelson, 2006; Zelder & Männel-Croisé, 2009).

The detection of analytes using chromogenic and fluorogenic chemosensors has been extensively employed because of the sensitivity, selectivity, and low cost of this technique. The wide variety of strategies which have been investigated has allowed the detection of analytes in different situations (Anslyn, 2004; Buske, Nicoleti, Cavallaro & Machado, 2015; Chhatwal, Kumar, Singh, Gupta & Awasthi, 2015; Heying, Nandi, Bortoluzzi & Machado, 2015; Houk, Wallace, Hewage & Anslyn, 2008; Nicoleti, Marini, Zimmermann & Machado, 2012; Nicoleti, Nandi & Machado, 2015; Pati, 2016; Snowden & Anslyn, 1999).

One type of system that has been widely explored in the recent years involves the use of polymeric materials with chromogenic or/and fluorogenic units anchored in their macromolecular structure, in order to achieve the selective detection of a particular analyte (Busschaert, Caltagirone, Van Rossom & Gale, 2015; Isaad & El Achari, 2011; Isaad, El Achari & Malek, 2013; Ma, Wu & Zeng, 2010; Machado, Stock & Reichardt, 2014; Sharma, Kaur, Singh, Kuwar & Singh, 2016; Trupp et al., 2010; Wu, Tang, Li & Li, 2015).

An attractive macromolecular material which can be employed as a support for the development of analyte sensing materials is ethyl(hydroxyethyl)cellulose (**EHEC**, **Fig. 1**) (Feller & Wilt, 1991; Kamide, 2005; Klemm, Heublein, Fink & Bohn, 2005). This modified cellulose can be easily functionalized for use in different applications (Isaad & El Achari, 2011; Karlberg, Thuresson & Lindman, 2005).

Recently, **EHEC** was functionalized with Brooker's merocyanine (**BM**) and the resulting polymer (**1a**), in a form of a film, was used in an acid–base strategy for anion sensing (Nandi, Nicoleti, Bellettini & Machado, 2014). **BM** is a classical merocyanine which has been extensively used as a perichromic dye (Cavalli, da Silva, Machado, Machado & Soldi, 2006; Koopmans & Ritter, 2007; Zimmermann-Dimer, Reis, Machado & Machado, 2009). System **1a** acts as chromogenic/fluorogenic system for the highly selective detection of CN^- in water (Nandi, Nicoleti, Bellettini & Machado, 2014). It was demonstrated that the polymer matrix acts as a support for anchoring the sensing units, resulting in a system with interesting features, such as the ability to detect CN^- in a highly selective fashion and in concentrations below the minimum established by the World Health Organization (WHO). The results obtained motivated us for the design of other related chromogenic polymeric systems for the detection of CN^- and even other anionic species in aqueous solution. Moreover, a comparison between related polymeric systems using different experimental procedures should provide further information about the microenvironment of the sensing units in the polymeric chains.

Thus, herein, we describe the preparation and characterization of two other modified **EHEC** polymers, obtained by the functionalization of **EHEC** with two merocyanine units with a molecular structure similar to **BM**, differing in terms of the replacement of the carbon−carbon double bond with carbon−nitrogen or nitrogen−nitrogen double bonds as conjugated bridges. The ability of the new systems to detect CN^- in water was compared with that of polymer **1a**. In addition, this study provided new evidence aimed at gaining a better understanding of the features responsible for the ability of the **EHEC** polymers to detect CN^- in water. The reuse of the films prepared was also evaluated.

2. Experimental

2.1. Reagents

All chemicals employed in this study were high−purity commercial reagents. The deionized water used in the measurements was boiled and bubbled with nitrogen and kept in a nitrogen atmosphere to avoid the presence of carbon dioxide. Tetra−*n*−butylammonium hydroxide was purchased from Sigma−Aldrich. All anions (HSO_4^-, $H_2PO_4^-$, NO_3^-, CN^-, CH_3COO^-, F^-, Cl^-, Br^-, and I^-) were used as tetra−*n*−butylammonium salts with purity greater than 97−99% and were dried over phosphorous pentoxide under vacuum before use. The anions were purchased from Fluka (F^-, > 97%; Cl^-, > 98%; NO_3^-, > 97%; $H_2PO_4^-$, > 97%), Vetec (Br^-, > 99%; I^-, > 99%; HSO_4^-, > 99%), and Sigma−Aldrich (CH_3COO^-, > 97%).

EHEC, Bermocoll E 230FQ lot no. 14770, was donated by Akzo Nobel Surface Chemistry AB, Stenungsund, Sweden. The degree of substitution of ethyl groups (DS_{ethyl}) in the polymer 0.9−1.0 per anhydroglucose unit and the molar substitution of ethylene oxide groups (MS_{EO}) is 1.9−2.2 per anhydroglucose unit. The molar weight of **EHEC**, determined by light scattering, is 3.30×10^5 g mol^{-1}. The polymer has a degree of polydispersity (M_w/M_n) below 2 (Dal Bó, Schweitzer, Felippe, Zanette & Lindman, 2005; Dal-Bó, Laus, Felippe, Zanette & Minatti, 2011; Modolon, Dal Bó, Felippe, Minatti & Zanette, 2009).

2.2. Instruments

UV−vis experiments were carried out on an HP 8452A spectrophotometer equipped with a thermostated bath and all measurements were performed at 25 °C, employing a 1 cm quartz cuvette. The films were fixed with adhesive tape on a quartz plate with 1 cm width × 4 cm height and subsequently introduced into the sample holder of the spectrophotometer. The spectrum was collected and the maximum wavelengths (λ_{max}) in the UV−vis spectra were calculated from the first derivative of the absorption spectrum. The λ_{max} values thus obtained were transformed into E_T(dye) values, according to the expression E_T(dye) = 28590/λ_{max} (Machado, Stock & Reichardt, 2014), given in kcal mol^{-1} with a precision of ±0.1 kcal mol^{-1}. Melting points were obtained on a Kofler hot stage and were uncorrected. The nuclear magnetic resonance (NMR) spectra were recorded on a Varian AS−400 spectrometer. Chemical shifts were recorded in ppm with the solvent resonance as the internal standard. Data are reported as follows: chemical shift, multiplicity (*s* = singlet, *d* = doublet, and *bs* = broad singlet), integration, and coupling constants (Hz). Infrared (IR) spectra were obtained on a Shimadzu model Prestige−21 spectrophotometer, with KBr pellets or silicon plates. In the latter case, the polymer was dropped in a 5% aqueous solution (wt/vol) onto the silicon plate and dried in an oven for 12 h at 30 °C before the analysis.

The thermal degradation of the films was analyzed using a Shimadzu TGA−50 thermogravimetric analyzer. The experiments were performed in the temperature range of 25−600 ºC at a heating rate of 5 ºC min^{-1}. The nitrogen flow was kept at 50 cm^3 min^{-1} and the analysis was performed in triplicate.

The time−resolved fluorescence experiment involving **1a** was carried out on an Easy life V^{TM} lifetime fluorometer using the stroboscopic technique (Optical Building Blocks Corp.). The fluorescence decay was obtained using a pulsed light from a diode (LED) of 445 nm as the excitation source and a long pass filter of 550 nm in order to isolate the emission of the film of polymer **1a** from the scattered light. The instrument response function (IRF) was measured using a Ludox scattering solution. Four scans were averaged for the fluorescence decay experiments. For every scan, the number of channels was 600, and the time of integration, over which the signal was averaged for every point of each scan, was 4 s.

The fluorescence decay curve for **BMH** in water was obtained using time−correlated single−photon counting (TCSPC). The pulsed excitation was at 400 nm, with a pulse duration of 150 fs, using a Ti:sapphire laser operating in locked mode, tuned at 800 nm, Mira−Coherent. The Mira laser oscillator was pumped by a Coherent Verdi 5W laser. The detection system used was a PMTMCP (Hamamatsu R3809U−50), cooled by the Peltier effect. The adjustment of the photomultiplier in this experiment was carried out employing a time increment of 48.83 ps, with the focal plane operating at −20 °C. The typical IRF was 50 ps at FWHM. The monochromator was set at 480 nm, close to the emission peak of the sample. The typical IRF was 50 ps at FWHM. The decays were recorded using TC900 and Edinburgh Instruments software and the curve was fitted using monoexponential analysis.

2.3. Synthesis of the compounds and the functionalized polymers

BM was prepared as described previously (Minch & Shah, 1977)

2.3.1. 4−((Pyridin−4−ylmethylene)amino)phenol (**8**)

4−Pyridinecarboxaldehyde (0.56 mL, 4.6 mmol) and 4−aminophenol (0.5 g, 4.6 mmol) were mixed, with magnetic stirring, in ethanol (5 mL). A drop of acetic acid was added and the reaction mixture was stirred for 4 h. A light green solid was formed, which was filtered under vacuum and recrystallized from ethanol. 72% yield. m.p. obtained: 207 ºC (literature (Demir, Kaya & Saçak, 2006): 210 ^{o}C). IR (KBr, $\bar{\nu}_{max}$/cm^{-1}): 3447 (ν, OH), 3063−2996 (ν, C−H), 1620, 1460 (ν, C=C), 1577 (ν, C=N), 1250 (ν, C−O). ^{1}H NMR (400 MHz, DMSO−d$_6$) δ/ppm: 9.69 (1H, *s*), 8.70 (2H, *d, J* = 6.1 Hz), 8.67 (1H, *s*), 7.80 (2H, *d*, *J* = 6.1 Hz), 7.30 (2H, *d*, *J* = 9.0 Hz), 6.83 (2H, *d*, *J* = 9.0 Hz). TGA: 231.75, 345.93, 470.73 ºC.

2.3.2. 4−(Pyridin−4−yldiazenyl)phenol (**9**)

4−Aminopyridine (1.5 g, 0.0159 mol) was dissolved in 7.5 mol L^{-1} hydrochloric acid (12 mL). A mixture containing 10% NaOH (10 mL), phenol (1.25 g, 0.0132 mol), and $NaNO_2$ (1 g, 0.014 mol) was then prepared and added dropwise at 8 °C to the 4−aminopyridine solution. The crude product was isolated by filtration and purified by crystallization from acetone/ethanol to give an orange powder (Agostini, Milani, Martínez-Máñez, Licchelli, Soto & Sancenón, 2012). 70% yield. m.p. obtained: 253 ^{o}C (literature (Buncel & Keum, 1983): 256 ^{o}C). IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 3550−3414 (ν, OH), 3043 (ν, C−H), 1640, 1580 (ν, C=C), 1468−1403 (ν, N=N), 1295 (ν, C−O). ^{1}H NMR (400 MHz, DMSO−d_6 + 2 drops of D_2O) δ/ppm: 8.92 (2H, *d, J* = 5.6 Hz), 8.14 (2H, *d, J* = 5.6 Hz), 7.96 (2H, *d*, *J* = 8.4 Hz), 7.01 (2H, *d*, *J* = 8.4 Hz). TGA: 262.96, 373.59 ^{o}C.

2.3.3. 4−(((4−Hydroxyphenyl)imino)methyl)−1−methylpyridin−1−ium iodide (**4a**)

Compound **8** (0.1 g, 0.5 mmol), iodomethane (0.03 mL, 0.5 mmol), and dry acetone (10 mL) were refluxed for 4 h and the flask was cooled in an ice bath. The brown solid formed was washed, filtered in cold acetone, and then recrystallized in ethanol. 80% yield. m.p. obtained: 223−225 ^{o}C (literature (Machado, Nascimento & Rezende, 1994): 225−227 ^{o}C). IR (KBr, $\bar{\nu}_{max}/cm^{-1}$): 3424 (ν, OH), 3153−3017 (ν, C−H), 1640, 1475 (ν, C=C), 1581 (ν, C=N), 1258 (ν, C−O), 1163 (δ, $C-N^{+}$). ^{1}H NMR (400 MHz, DMSO−d_6) δ/ppm: 10.00 (1H, *s*), 9.04 (2H, *d, J* = 6.6 Hz), 8.95 (1H, *s*), 8.43 (2H, *d*, *J* = 6.6 Hz), 7.46 (2H, *d*, *J* = 8.6 Hz), 6.88 (2H, *d*, *J* = 8.6 Hz), 4.35 (3H, *s*). TGA: 244.91, 373.90, 522.41 ^{o}C.

2.3.4. 4−((4−Hydroxyphenyl)diazenyl)−1−methylpyridin−1−ium iodide (**5a**)

Compound **5a** was prepared following the procedure described in the literature (Buncel & Keum, 1983; Garcia-Amoros, Nonell & Velasco, 2012). Compound **9** (0.5 g, 2.5 mmol) was refluxed with iodomethane (12.5 mmol) for 4 h in a mixture of acetone/tetrahydrofuran (7:3 v/v). The orange solid formed was washed and filtered in cold acetone. 80% yield. m.p. obtained: 217−220 ºC (literature (Buncel & Keum, 1983): 220−222 ºC). IR (KBr, $\bar{\nu}_{max}$/cm^{-1}): 3550−3414 (ν, OH), 3124−3055 (ν, C−H), 1633, 1582 (ν, C=C), 1411 (ν, N=N), 1295 (ν, C−O), 1146 (δ, C−N^{+}).^{1}H NMR (400 MHz, DMSO−d$_6$) δ/ppm: 11.38 (1H, *s*), 9.10 (2H, *d, J*= 4.5 Hz), 8.27 (2H, *d, J* = 4.5 Hz), 7.97 (2H, *d*, *J* = 7.0 Hz), 7.06 (2H, *d*, *J* = 7.0 Hz), 4.36 (3H, *s*).

2.3.5. Preparation of the polymers functionalized with the sensing units

Polymers **1a**−**3a** were prepared using a tosylated **EHEC** (**6**) as the intermediate, for which the synthesis was previously described together with that of polymer **1a** (Nandi, Nicoleti, Bellettini & Machado, 2014).

The film formed from **6** was refluxed in 250 mL of ethanol with 0.3 g (1.55 mmol) of compound **8** for 12 h to give **2a**. The resulting film was then washed five times with 100 mL of ethanol resulting in a yellow colored film. IR (silicon wafer, $\bar{\nu}_{max}$/cm^{-1}): 3433 (ν, OH), 2972−2874 (ν, C−H), 1640−1470 (ν, C=C), 1634 (δ, OH), 1157 (δ, C−N^{+}). TGA: 239.44, 299.29 ºC. The same procedure was used for the synthesis of **3a**, by means of the reflux of **6** with compound **9**. IR (silicon wafer, $\bar{\nu}_{max}$/cm^{-1}): 3430 (ν, OH), 2972−2871 (ν, C−H), 1099 (δ, C−N^{+}). TGA: 317.63, 365.91, 571.09 ºC.

2.4. pK$_a$ determinations

A solution of **4a** or **5a** was prepared in a concentration of 4×10^{-3} mol L^{-1} in acetone, and stored in glass flasks closed with rubber stoppers to avoid the evaporation of the solvent. An aliquot of the solution, sufficient to give a concentration of the compounds of 2×10^{-5} mol L^{-1}, was collected with a microsyringe and placed in a flask. After the evaporation of the solvent, distilled water was added, the pH was adjusted with the addition of small aliquots of HCl (0.1 mol L^{-1}) or KOH (0.1 mol L^{-1}), and the UV−vis spectrum of the compound was recorded at 25 ºC. The pH of the solution was measured and the UV−vis spectrum was obtained. Data were fitted using a sigmoid equation to give the pK_a value.

The pK_a values were also determined for the films of **EHEC** with the anchored chemosensors. In these experiments, the film was immersed in 10 mL of distilled water in vials, with the pH previously adjusted by the addition of small aliquots of HCl (0.1 mol L^{-1}) or KOH (0.1 mol L^{-1}). The UV−vis spectrum of the film was recorded at 25 °C after 3 min of immersion. Data were fitted using a sigmoid equation to give the pK_a value.

2.5. Influence of pH on the detection of cyanide

To determine the pH dependence on the CN^- detection, using films of **1a** and **2a**, various CN^- aqueous solutions (6.0×10^{-4} mol L^{-1}) were prepared at different pH values in vials containing 10 mL of distilled water with the pH previously adjusted with HCl solutions (0.1 mol L^{-1}) or KOH (0.1 mol L^{-1}). The films were immersed into these solutions and after 5 min the UV−vis spectra were collected and compared with the spectrum of the film in an aqueous solution of tetra−*n*−butylammonium hydroxide (6×10^{-4} mol L^{-1}). A bar graph of the concentration percentage in relation to the real CN^- value as a function of pH was constructed.

2.6. Determination of the apparent concentration of the sensing units in the films

To determine the concentration of compound **4a** anchored in the film of **2a**, a calibration curve was obtained using **4a** as the standard in DMSO (**Fig. S19**). A linear fit of the experimental data gave the equation: Absorbance = $(1.55\pm0.01)\times10^4$ c (**4a**), with $R^2 = 0.9999$ and S.D. = 1.0×10^3. Subsequently, a film of **2a** (1.5×10^{-4} g) was dissolved in DMSO (5 mL) and the maximum absorbance value (at 455 nm) was collected, this being 0.30, allowing the apparent concentration of **4a** covalently bonded to the film to be estimated with the use of the calibration curve, which was calculated as c (**4a**) = 1.93×10^{-5} mol L^{-1} or 2.18×10^{-3} g of **4a** per g of **EHEC**. This methodology was also used in the determination of the apparent concentration of compound **5a** (**Fig. S20**) in the film of **3a** (1.5×10^{-4} g dissolved in 5 mL of DMSO). A linear fit of the experimental data gave the equation: Absorbance = $(1.56\pm0.018)\times10^4$ c(dye), with $R^2 = 0.994$ and S.D. = 4.8×10^2. The value of the absorbance found at 409 nm was 0.78, providing c (**5a**) = 1.56×10^{-5} mol L^{-1} or 5.7×10^{-3} g of **5a** per g of **EHEC**.

*2.7. Titration experiments using the film of **2a** with cyanide and determination of limits of detection and quantification*

Titration experiments were performed in water (adjusted to pH = 8.0) through the immersion of the film of **2a** (5 min) in flasks with different concentrations of tetra−*n*−butylammonium cyanide. UV−vis spectra were obtained and the absorbance values were collected at 498 nm.

The linear segment of the titration curve was used to estimate the optical detection limit (*DL*) and the optical quantification limit (*QL*). Based on the linear fit of the experimental data, the *DL* and *QL* were obtained from equations (1) and (2) according to a procedure reported in the literature (Isaad, El Achari & Malek, 2013; Skoog & West, 1982), where Sb_1 is the standard deviation of the blank solution and S is the slope of the calibration curve. The B values are 3 for *DL* and 10 for *QL*.

$$DL = B\,Sb_1 / S \qquad (1)$$

$$QL = B\,Sb_1 / S \qquad (2)$$

3. Results and Discussion

*3.1. Functionalization of **EHEC***

The decorated polymers **1a−3a** were prepared as shown in **Scheme 1**. The tosylation of **EHEC**, in the first step, was carried out using a previously described methodology (Nandi, Nicoleti, Bellettini & Machado, 2014). In the second step, the film of the tosylated intermediate **6** was reacted with compound **8** or **9** to yield the functionalized **EHEC** products. The films have different colors depending on whether the anchored sensing units are protonated or deprotonated. The deprotonation was easily accomplished by dipping the films into aqueous solutions of tetra−*n*−butylammonium hydroxide.

3.2. Characterization

The synthetically−modified polymers were characterized using the IR technique. The characterization of **6** and polymer **1a** has been previously reported (Nandi, Nicoleti, Bellettini & Machado, 2014). With the reaction of **6** with **8**, the bands relative to the tosyl group disappeared in the IR spectrum of the resulting polymer **2a** (**Fig. S5**). In addition, the appearance of the bands relative to the anchored **4a** unit (see **Fig. S3** for comparison) could be observed at 1643−1470 cm^{-1} (ν, C=C$_{\mathrm{arom}}$) and 1157 cm^{-1} (δ, C−N^{+}). These data are consistent with values reported in the literature (Alsaygh, Al-Humaidi & Al-Najjar, 2014; Demir, Kaya & Saçak, 2006; Xu, Ling & He, 2008). Polymer **3a** was similarly characterized and the bands relative to the anchored **5a** unit (**Fig. S10**) could be observed at 1099 cm^{-1} (δ, C−N^{+}).

Data obtained from the thermogravimetric (TG) curves (mass versus degradation temperature) and the corresponding derivative thermogravimetric (DTG) curves (mass loss rate versus temperature) were used to assess the effect of the functionalization on the degradation pattern of the **EHEC** film. The TG/DTG curves are provided in the Supporting Information. For the **EHEC** film the maximum degradation temperature (T_{max}) is 375.3 ºC, while for the film of tosylated EHEC (**6**) the T_{max} value is 213.0 ºC (Nandi, Nicoleti, Bellettini & Machado, 2014). For the film obtained from **2a** values of 239.44 and 299.20 °C were obtained (**Fig. S13**). For comparison, for a film prepared with a physical mixture of **EHEC** and **4a** (which corresponds to the same sensing unit in functionalized **2a**), T_{max} value is 367.4 ºC (**Fig. S14**), which differs only slightly from that obtained for the **EHEC** film. The same pattern was found for the polymer **3a**, with T_{max} values of 317.63 and 365.91 ºC (**Fig. S17**), while a film obtained from the physical mixture of **EHEC** and the corresponding azophenol **5a** exhibits T_{max} = 372.4 ºC (**Fig. S18**), which is very similar to the **EHEC** film value (Nandi, Nicoleti, Bellettini & Machado, 2014). These studies showed that significant changes in T_{max} only occur when the **EHEC** film is chemically modified to generate the functionalized polymers **2a** and **3a**.

Spectrophotometric measurements involving a comparison of dyes **4a** and **5a** in DMSO solution with the polymers **2a** and **3a** allowed the apparent concentration of each sensing unit anchored in **EHEC** to be calculated. For **2a**, *c* (**4a**) was 1.93×10^{-5} mol L^{-1} or 2.18×10^{-3} g of dye/g of **EHEC**, while the value determined for **5a** in the film of **3a** was *c* (**5a**) = 1.56×10^{-5} mol L^{-1} or 5.7×10^{-3} g of dye/g of the polymer.

The films of **2a** and **3a** were immersed in an aqueous solution of tetra−*n*−butylammonium cyanide and it was verified that the sensing units chemically anchored in the polymer matrix were not released into the aqueous solution (**Figs. S21A** and **S22A**). In contrast, when a film prepared from a physical mixture of **4a** and **EHEC** (or **5a** and **EHEC**) was immersed in an aqueous solution of tetra−*n*−butylammonium cyanide the solution quickly became colored, indicating that the dyes are lixiviated to the aqueous solutions (**Figs. S21B** and **S22B**).

3.3. Studies on pK_a of the functionalized polymers

Plots of the maximum absorbance as a function of the pH for compound **4a** in water and for the film of **2a** immersed in water were used for the determination of the pK_a values for the phenols at 25 °C (**Fig. S23 a, b**). Similar experiments were performed for compound **5a** and for the film of **3a** (**Fig. S23 c, d**). The pK_a values for **BMH** and **1a** have been previously reported (Davidson & Jencks, 1969; Nandi, Nicoleti, Bellettini & Machado, 2014). All pK_a values are given in **Table 1**. The pK_a value for the sensing units in **2a** was 9.75±0.04. This value is higher than that for free **4a** in aqueous solution (pK_a = 9.00±0.03). These trends are very similar to those reported for **BM** and polymer **1a** and for **5a** in comparison with the film of **3a**. Thus, all data show that the acidity of the phenols is decreased if their microenvironment is changed from water to the polymeric chains. It is well known that the more acid the phenol, the lesser the basicity of its conjugated base, being this decrease associated with stereoelectronic effects and/or with the ability of the medium in stabilizing the conjugated bases by solvation. The higher pK_a values verified for the sensing units anchored in the polymers, in comparison with the values for the corresponding compounds in water, suggest that the microenvironment of the sensing units in the films are protected from the bulk water.

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3.4. Perichromic studies on the polymer films

Table 2 shows the molar transition energy (E_T) values for the vis perichromic bands of **BM**, **4b**, and **5b** in various solvents, as well as for the films of **1b–3b** immersed in aqueous solution. The deprotonation of **4a** in aqueous solution leads to the appearance of the solvatochromic band at λ_{max}= 476.0 nm. Interestingly, the deprotonation of the phenol units in the film of **2a** led to the appearance of the perichromic band corresponding to the dye units at λ_{max} = 498.0 nm. This bathochromic shift of $\Delta\lambda_{max}$= +22 nm suggests that the dye is in a less polar environment in the film (Machado, Stock & Reichardt, 2014). **Fig. 2** shows solutions of **BM**, **4b**, and **5b** in some solvents, as well as the films of **1b−3b** after immersion in water. The film of **2b** in aqueous medium has a similar color to the dye **4b** in methanol, a less polar solvent than water, i.e., the environment of the dye in the film has a polarity very similar to that of methanol. For the film of **3b**, the dye units in the film are responsible for the perichromic band at λ_{max} = 540.0 nm. The corresponding dye **5b** in aqueous solution has a solvatochromic band with λ_{max}= 530.0 nm and a bathochromic shift of $\Delta\lambda_{max}$= +10 nm. The film of **1b** has a similar behavior, as previously reported (Nandi, Nicoleti, Bellettini & Machado, 2014).

*3.5. Fluorescence decay curve for **1a***

Fluorescence decay curves for the film of **1a** and for **BM** (**Fig. S24**) were fitted using single−exponential analysis. The fluorescence lifetime of **BMH** in water was determined as 0.030 ns (χ^2 = 1.351), while the lifetime observed for the film of **1a** was 0.85±0.01 ns (χ^2 = 0.9). The increase (by a factor of >28) in the lifetime value suggests that the hydrocarbonic chains in **1a** provide hydrophobic microenvironments and increase the rigidity of the system, which leads to a decrease in the non−radiative transitions of the **BMH** units.

*3.6. UV−vis study of **2a** and **3a** with the anions*

Fig. 3A shows films of **2a** before and after immersion in aqueous solutions of various anions. The film of **2a** is yellow and that of **2b** (obtained after immersion in an aqueous HO^- solution) is red. Of the several anion solutions studied, only CN^- is able to change the color of the film to red, which is sufficiently basic to deprotonate the dye in aqueous solution, forming **2b**. **Fig. 3B** shows films of **3a** before and after immersion in aqueous solutions of various anions. The film of **3a** is yellow and that of **3b** (obtained after immersion in an aqueous HO^- solution) is pink. The very low pK_a value of **5a** influences the selectivity and precludes the use of this compound in the detection of CN^- in aqueous media.

Fig. 4 shows the UV−vis spectrum for the film of **2a**, which exhibits a band with maximum wavelength λ_{max} = 408 nm. This band disappears with the immersion of the film in an aqueous solution of CN^- simultaneously with the appearance of another band with λ_{max} = 498 nm, while the other anions studied did not cause any spectral alteration.

The results obtained in the experiments allowed us to verify that the films of **1a** are applicable for the detection of CN$^-$ in water within a pH range of 7.4−8.8 (**Fig. 5A**) while the film of **2a** detects CN$^-$ at pH 8.0−8.5 (**Fig. 5B**). Optical chemosensors based on acid-base processes require attention with the results obtained since these strategies are sensitive to changes in pH, which could lead to false positives (Schramm, Nicoleti, Stock, Heying, Bortoluzzi, & Machado, 2017).

The recyclability of the systems was verified by immersing the films alternately in CN$^-$ and in acid aqueous solutions. The film of **1a** was reused several times after appropriate treatment (**Fig. 5C**), but the film of **2a** was only reused twice after being washed with a slightly acidic aqueous solution (**Fig. 5D**). The limitation in the reuse of the film of **2a** is probably due to the hydrolysis of the double carbon−nitrogen bond (Heying, Nandi, Bortoluzzi & Machado, 2015) of the sensing unit.

*3.7. Titration of **2a** with cyanide*

Fig. 6A shows a plot of absorbance values at λ_{max} = 498.0 nm for the film of **2a** as a function of c(CN$^-$), which suggests a behavior typical of a 1:1 stoichiometry, considering the anion and the dye units anchored in the polymer. The experimental data were fitted with the use of equation (3) (Buske, Nicoleti, Cavallaro & Machado, 2015; Nicoleti, Marini, Zimmermann & Machado, 2012; Valeur, Pouget, Bourson, Kaschke & Ernsting, 1992), which is related to the following situation according to a 1:1 chemosensor:anion stoichiometry.

$$Abs = [Abs_o + Abs_{11}K_{11}C_{A-}] / [1 + K_{11}C_{A-}] \qquad (3)$$

In this equation, Abs is the absorbance value after each addition of an anion, Abs_0 is the initial absorbance without an anion added, Abs_{11} is the maximum absorbance value obtained with the addition of an anion considering a 1:1 **1a**:anion stoichiometry, C_{A-} is the anion concentration for each addition, and K_{11} is the equilibrium constant. A fitting of the experimental data provided an equilibrium constant of $(3.72\pm0.55)\times10^{3}$ L mol^{-1}. An analysis of the linear segment of the titration curve (**Fig. 6B**) gave $DL = 9.36\times10^{-6}$ mol L^{-1} and $QL = 3.12\times10^{-5}$ mol L^{-1} for the detection of CN^{-} in aqueous solution.

4. Conclusions

The functionalization of **EHEC** with the compounds **7–9** allowed the obtainment of polymers **1a–3a**. Films of **1a** and **2a** can be used for the highly selective chromogenic (and in the case of **1a** also fluorogenic) detection of CN^{-} in water. The role of the polymeric chain, protecting the sensing unit from the medium, explains the ability of this ensemble to sense very small concentrations of CN^{-} in water, which is not possible if the corresponding protonated dyes are used in water.

The fluorescence lifetime obtained for the film of **1a** suggests that the fluorophore is protected from the bulk water, being located in hydrophobic microenvironments in the polymer, leading to an increase in the rigidity and to a lowering of the non-radiative transitions.

For the film of **2a** the stoichiometry between the anion and dye anchored to the film was determined by UV−vis techniques as being a 1:1 ratio, i.e., the CN^{-} interacts with the phenol group of the dye molecule, with deprotonation occurring and a subsequent change in the color of the film.

Studies have shown that **1a** can be used several times, while the film of **2a** has a limited number of uses (two cycles), this limitation being due to the hydrolysis of the imine bond (Heying, Nandi, Bortoluzzi & Machado, 2015) in aqueous medium. The low pK_a value obtained for the film of **3a** hinders its use in the detection of CN^- in water.

Polymeric films **1a** and **2a** are promising for the development of optical devices for the detection CN^- in water with low detection limits. Such films can also be used as inspiration models for the development of new detection systems with various potential analytical applications.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/

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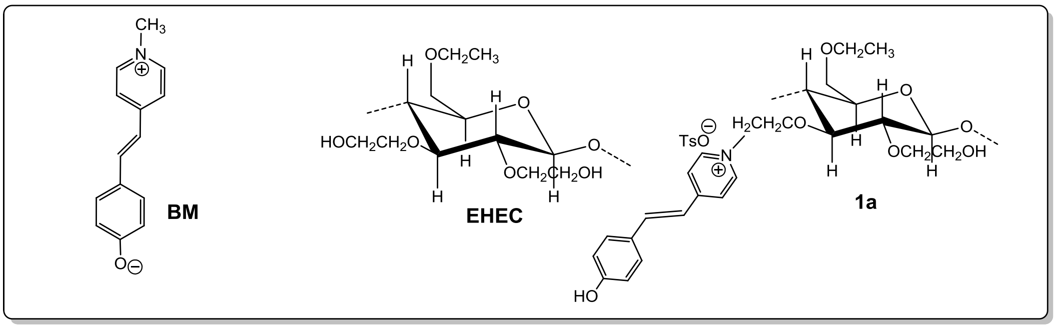


Fig. 1. Structures of BM, EHEC, and 1a.

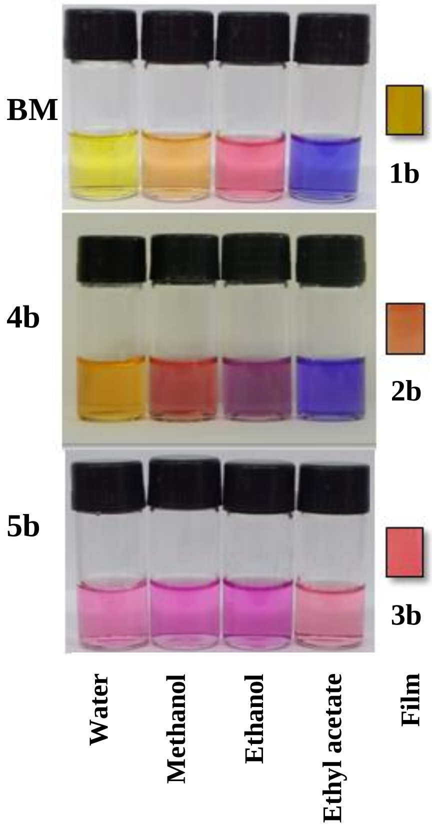


Fig. 2. Solutions of BM, 4b, and 5b in water, methanol, ethanol, and ethyl acetate. The films of 1b−3b after immersion in aqueous solution of tetra−*n*−butylammonium hydroxide are also presented.

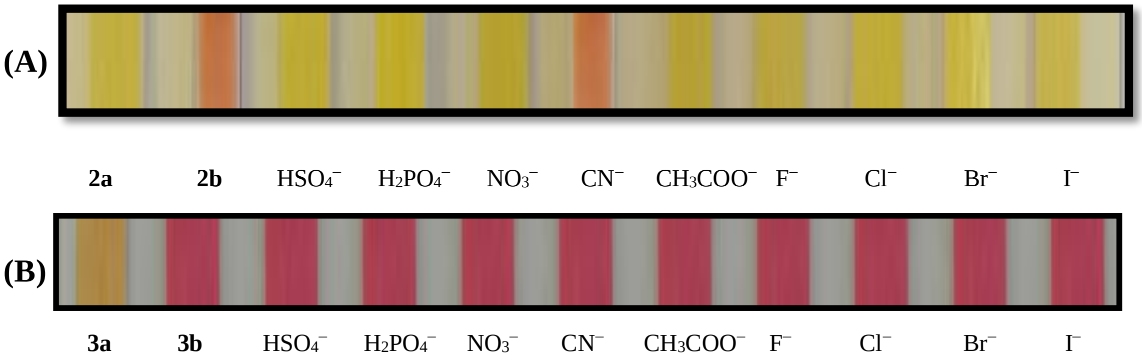


Fig. 3. Films of (A) 2a or (B) 3a after immersion in aqueous solutions containing HSO_4^-, $H_2PO_4^-$, NO_3^-, CN^-, CH_3COO^-, F^-, Cl^-, Br^-, and I^- [*c* (anion) = 6.0×10^{-4} mol L^{-1}].

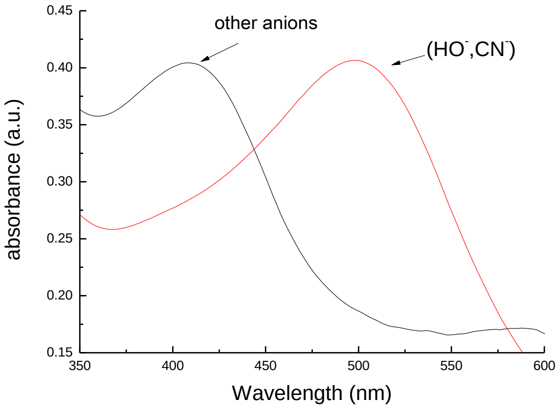


Fig. 4. UV−vis spectra for films of 2a in the presence of HSO_4^-, $H_2PO_4^-$, NO_3^-, CN^-, CH_3COO^-, F^-, Cl^-, Br^-, HO^-, and I^- in water [*c* (anion) = 6.0×10^{-4} mol L^{-1}].

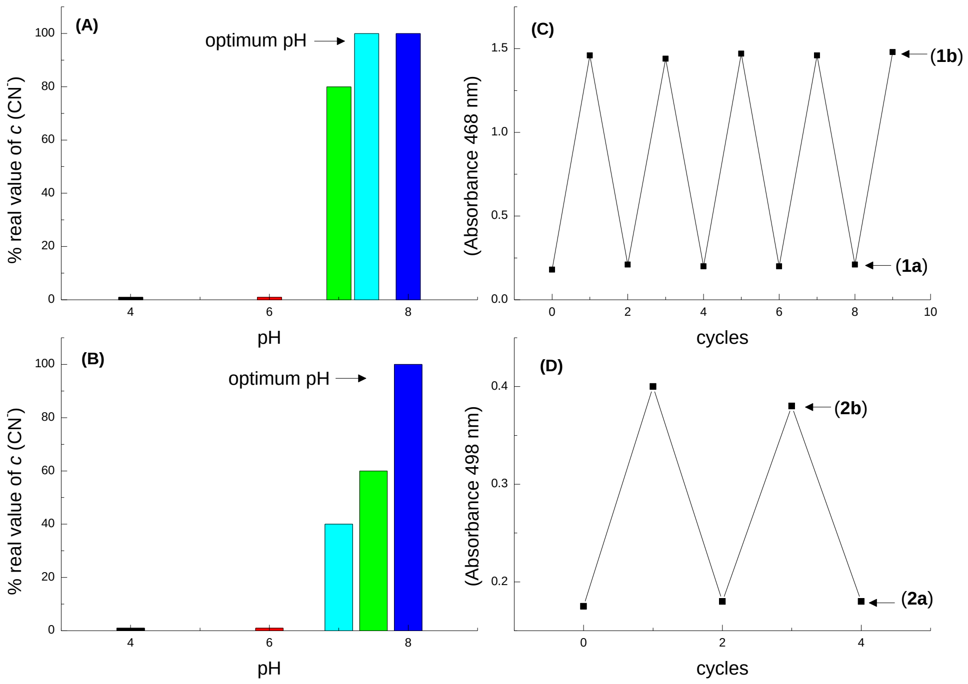


Fig. 5. Influence of pH on the determination of CN^- using films of (A) 1a and (B) 2a. Reversibility of the films of (C) 1a and (D) 2a, verified by the alternate immersion of the polymers in CN^- and acid aqueous solutions.

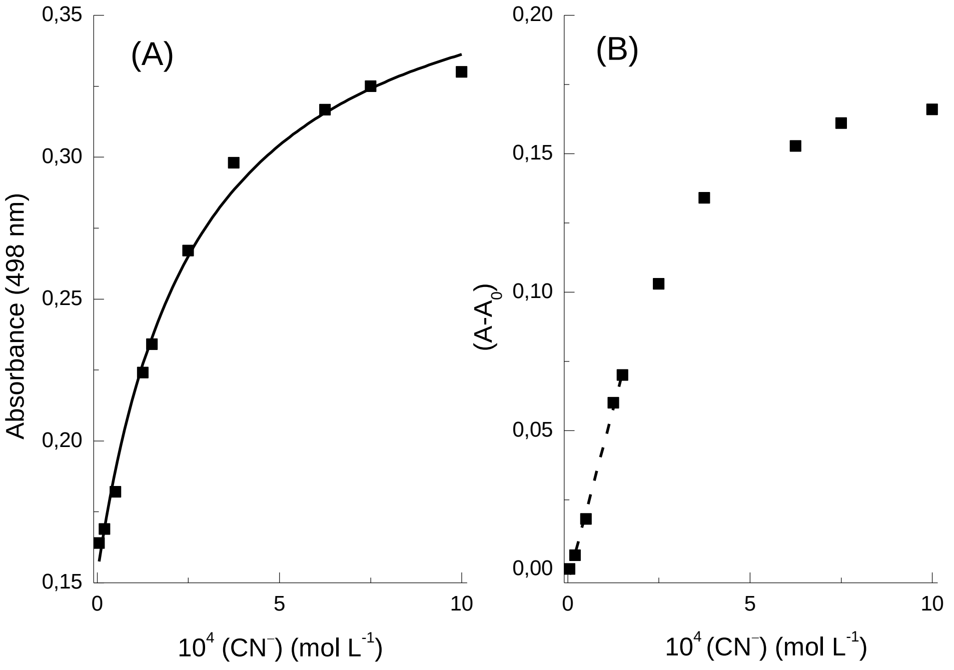
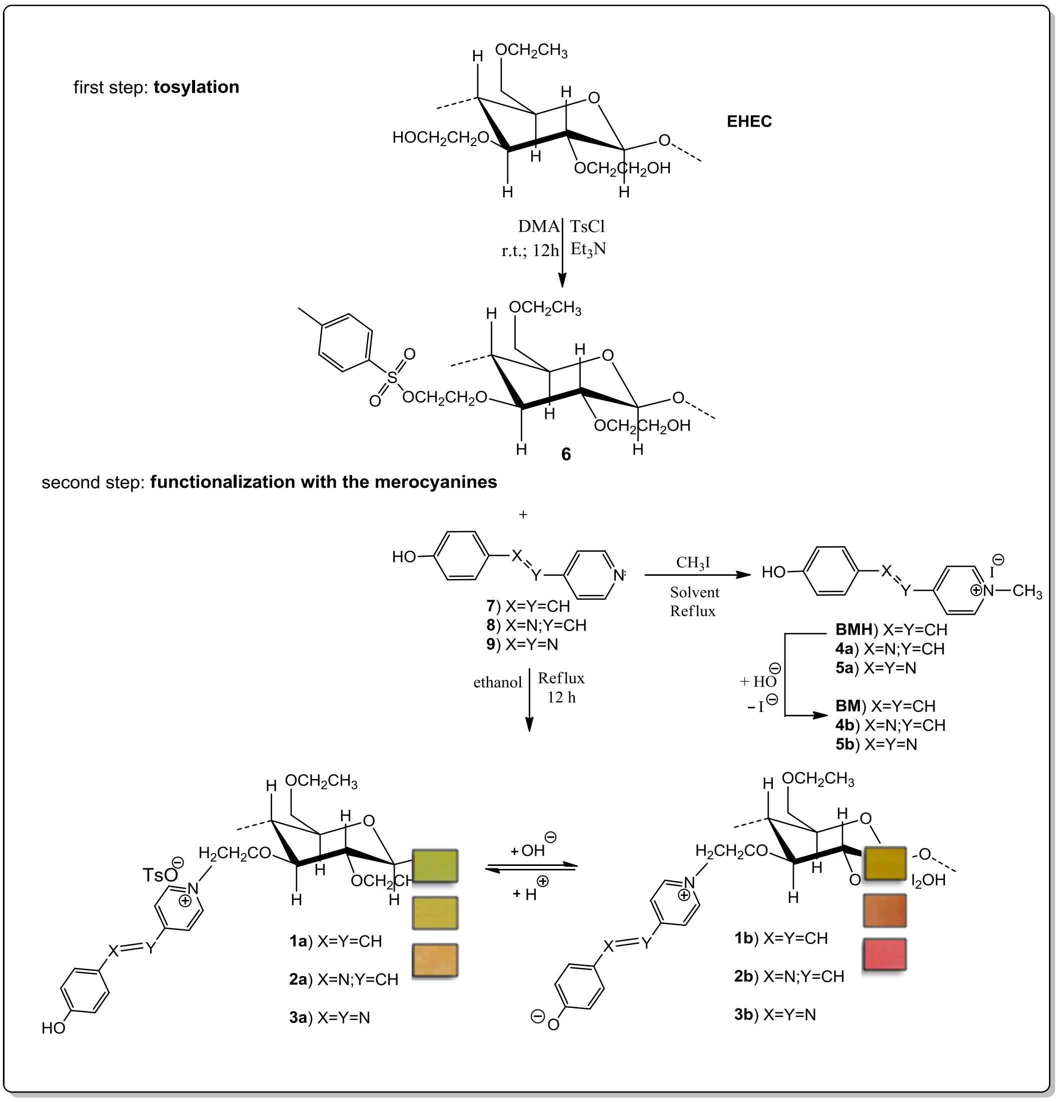


Fig. 6. (A) Curve of the variation in the absorbance at 498 nm of the film of 2a with the addition of increasing amounts of CN$^-$. (—) fitting of experimental data to determine the binding constant. (B) Normalized titration curve used to determine *DL* and *QL*. (- - -) Linear fitting of the experimental data for the determination of *DL* and *QL*.



Scheme 1. Synthesis of the functionalized polymers **1a**–**3a** and of dyes **BM**, **4b**, and **5b**.

Table 1. pK_a values for **BMH**, **4a**, and **5a** at 25 °C and for the films of polymers **1a**-**3a**.

Compound	pK_a	Polymer	pK_a
BMH[a]	8.6	**1a**[b]	9.84 ± 0.02
4a	9.00±0.03	**2a**	9.75± 0.04
5a	5.85±0.01	**3a**	6.01± 0.09

[a] From (Davidson & Jencks, 1969). [b] From (Nandi, Nicoleti, Bellettini & Machado, 2014)

Table 2. Molar transition energy (E_T) values for BM, 4b, and 5b in various solvents and for the films of 1b−3b (immersed in aqueous solution of tetra−*n*−butylammonium hydroxide with a concentration of 6×10^{-4} mol L^{-1}).

	E_T (kcal mol^{-1})		
Solvent	E_T (**BM**)	E_T (**4b**)	E_T (**5b**)
1,2−dichloroethane	48.7	44.5	54.4
DMA	48.5	45.7	52.4
DMSO	49.7	46.3	51.8
acetonitrile	51.0	48.0	53.5
ethyl acetate	49.6	50.0	55.7
ethanol	55.7	52.2	51.8
methanol	59.0	55.8	52.5
water	64.5	60.1	53.9
Film[a] in water	61.1 (**1b**)	57.4 (**2b**)	52.9 (**3b**)

[a] Polymer in parentheses