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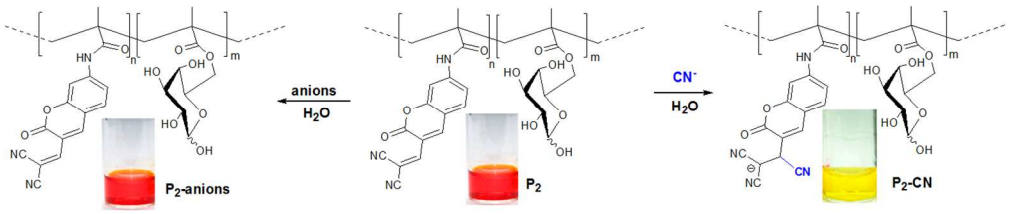

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ARTICLE TYPE

Water soluble and fluorescent copolymer for highly sensitive and selective fluorescent chemosensor for cyanide anion detection in biological medium

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We report a simple colorimetric method based on water soluble polymer (WSP) for detection of cyanide sensitively and selectively in biological solution. The water soluble polymers WSP were prepared from a radical polymerization of a methacrylate with cyanide chemosensor moiety based on a coumarin - dicyano-vinyl derivative motif, and the polymer water solubility was achieved by copolymerizing this hydrophobic monomer with others glyco-conjugated methacrylate derivatives. The lowest concentration for quantification of cyanide ions was 0.05 µM, and other common anions nearly have no colorimetric response. Therefore, the chemical incorporation of some carbohydrates as lactose or its usual monosaccharide's moieties (glucose, lactose) represents a new way for the implementation of the polymers for the cyanide anions detection in water selectively and efficiently.

Introduction

Considerable efforts have been put into the design and synthesis of functional molecules that serve as probes or sensors for the detection of chemically and biologically important ionic species.[1,2] Recent studies showed that some toxic anions also act as severe environmental pollutants and have adverse health effects. [3-6] One of the most rapidly acting and powerful poisons is cyanide CN−. It strongly binds the active site of cytochrome-c and inhibits the mitochondrial electron-transport chain, leading to decreased oxidative metabolism and oxygen utilization.[7,8] The maximum permissive level of cyanide in drinking water is therefore set at 1.9 µM by the World Health Organization (WHO). [9,10] Given its acute toxicity, wide availability in massive amounts, and in light of increasing terrorist activity, there is a pressing need for fast, accurate detection of cyanide at the regulated concentrations in aqueous mediums. Various methods used previously to analyze cyanide employ titrimetric,[11] voltammetric,[12] potentiometric[13] and electrochemical methods[14] as well as ion chromatography.[15] However, these methods often require extensive, time consuming procedures that involve the use of sophisticated instrumentation with high detection limits. Optical sensors for cyanide, in which a change in color and/or fluorescence intensity (or emission wavelength) is monitored, have been studied actively over the past ten years due to their simple, inexpensive, and rapid implementation. Generally, three approaches for chemosensors [16]: (a) the binding site-signaling subunit,[17-19] (b) the chemodosimeters,[20-33] or (c) the displacements[34–40] has been extensively reported. However, these chemosensors suffer from several problems: they (i) act only in pure organic solvents or solutions containing a large amount of organic solvents; (ii) show poor selectivity to CN− and, (iii) show high detection limit. In the recent years, cyanide optical chemosensors that operate in aqueous solutions are developed, [41-45] and in our laboratory we developed a novel water soluble chemosensors [46-52] behaves as a colorimetric cyanide receptor in water at room temperature. The chemical structure of these water soluble chemosensors is taken from the structure of our new glycoconjugated dyeing agents developed recently.[53-59] The water solubility of these new chemosensor generation was given by the incorporation of the saccharidic, [46-48] glycerol[49], moiety on the starting organic chemosensor or by the incorporation of the starting chemodosimeters on an adequate water soluble polymer as polyvinyl alcohol,[50] on a natural cellulose materials,[51] through a chemical grafting or also by the organic chemosensor incorporation into polymeric plastic film based on starch as a biosourced biopolymer.[52] One the other hand, it should be noted that the area of responsive water soluble polymers (WSP) has nowadays evolved well beyond the demonstration of novel and interesting properties. Currently, the exploitation of useful and advanced functions such as drug or gene carriers with triggered release properties, catalysis, detection and imaging, environmentally adaptive coatings, and self-healing materials has emerged to be a more relevant subject. The capability of facile manipulation of the solubility, hydrodynamic volume, and chain configuration and conformation of responsive polymers by external stimuli has indeed enabled the development of responsive polymeric systems with novel functions. Intuitively, responsive polymers should play an important role in detection and sensing applications. However, stimuli-responsive polymer-based detection systems are still in its infancy stage as compared to the relatively mature field of small molecule probes.[17-40] In this paper, we designed and synthesized a novel glycoconjugated poly

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coumarin-cyanocarbon (P2) containing coumarin-dicyano-vinyl unit in its main chain as a highly selective and sensitive cyanide sensor and a carbohydrate, such as galactose as a water soluble moiety (figure 1). Its design is based on the consideration that the dicyano-vinyl group can act as a selective cyanide-reactive unit for the nucleophilic addition reaction. Meanwhile, because of the water solubility property of the WSP, the solvated cyanide ions to reach the guest or the transducer cores, giving rise to the sensing phenomenon, resulting in higher sensitivity toward cyanide detection

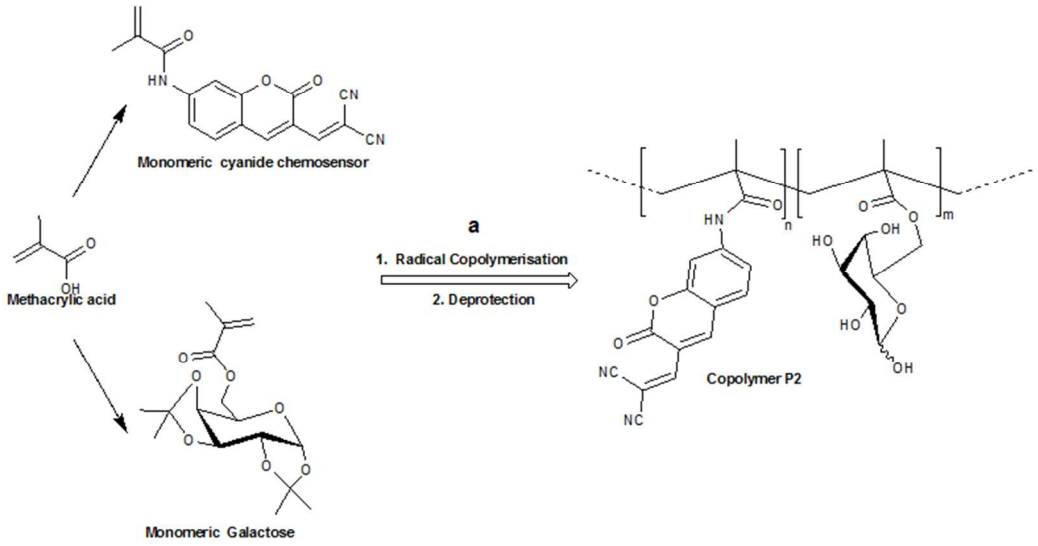
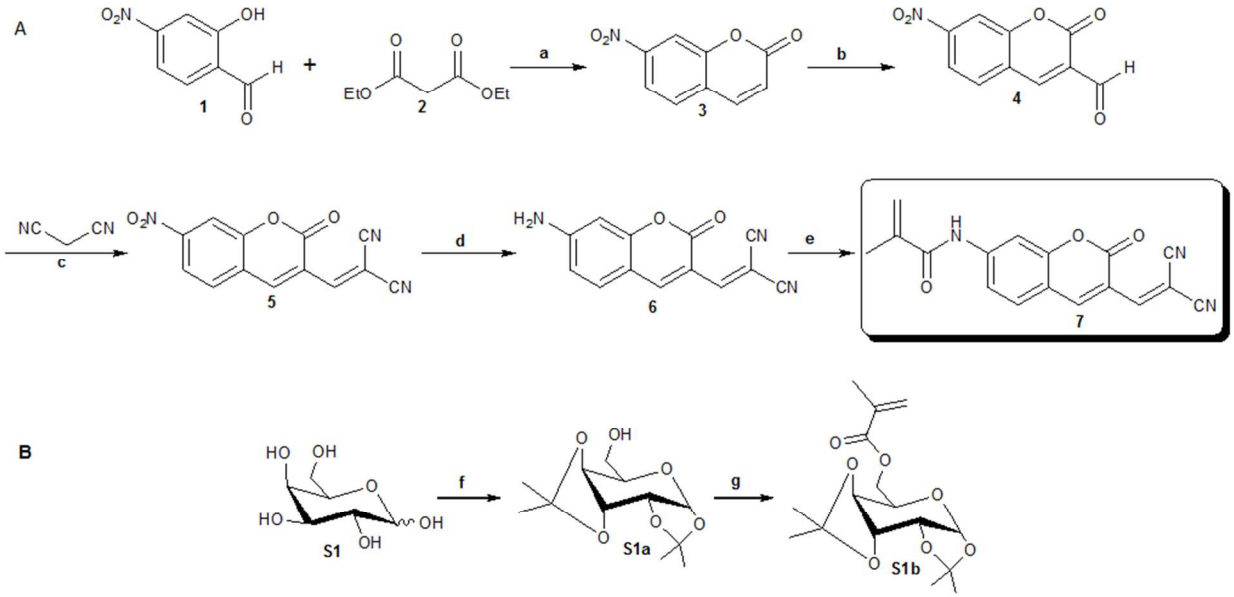


Figure 1: Chemical structure of the monomers and the copolymer P2

2 Results and discussion

2.1 Monomer synthesis and characterization

As mentioned in the introduction, we hypothesized that the chemical anchoring of a sensing motif to a hydrophilic polymer chain could provide a water-rich environment to the sensing moiety upon polymer swelling, thus permitting the solvated cyanide ions to reach the sensory receptor, giving rise to the sensing phenomenon, resulting in cyanide detection in water. For this purpose, methacrylic monomer containing a coumarin-dicyano-vinyl derivative (7) as a colorimetric cyanide chemosensor and glyco-conjugated methacrylic monomers (S1b) were prepared as reported in scheme 1.



Scheme 1: Synthesis of the monomers. Reagents and conditions: (a) $Na_2CaP_2O_7$, EtOH/H_2O, RT, 1h (b) $POCl_3$, DMF, 50°C, 30 min (c) $Na_2CaP_2O_7$, EtOH/H_2O, RT, 1h (d) $SnCl_2$, MeOH, reflux, 4h (e,g) THF, methacrylic chloride, RT, 1h (f) 1. DMP, TsOH, 80°C, 8h, 2. NEt_3, 15 min, 3. H_2O/MeOH, 80°C, 1h.

Compounds **3** and **5** were prepared by Knoevenagel condensation of 2-hydroxy-4-nitrobenzaldehyde**1** with diethyl malonate**2** in the case of the compound **3** and 7-nitro-2-oxo-2H-chromene-3-carbaldehyde **4** with malononitrile in the case of the compound **5**, in the presence of $Na_2CaP_2O_7$ as a basic catalyst. The use of $Na_2CaP_2O_7$ as heterogeneous catalyst in the

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Knoevenagel condensation has allowed the isolation of the compounds **3** and **5** rapidly (20 min for **3** and 15 for **5**) and with good yield (about 94%). The addition of water (5% in EtOH) remarkably decreases the reaction time of the $Na_2CaP_2O_7$ in the Knoevenagel synthesis of the compounds **3** and **5.** In fact, the time reaction goes from 1h to 20 min and from 40 to 15 min in EtOH for **3** and **5** respectively. The $Na_2CaP_2O_7$ was regenerated, by calcinations at 500°C during 15 min, and after seven successive recuperations. Then **3**was treated by $POCl_3$ chloride in DMF to give the compound **4**. The compound **6** was obtained by the reduction of the nitro group in the presence of $SnCl_2$ inMeOH and under reflux. In the final step, and in order to prepare the corresponding monomors **7** and $\mathbf{S_{1b}}$, the compounds **6** and $\mathbf{S_{1a}}$ were reacted with methacryloyl chloride in THF solution and at room temperature to afford the corresponding methacrylate monomers. All intermediates and monomers were characterised by ^1H and ^{13}C NMR spectroscopy. Figure 2 reports the ^1H NMR spectra of the monomer **7**.

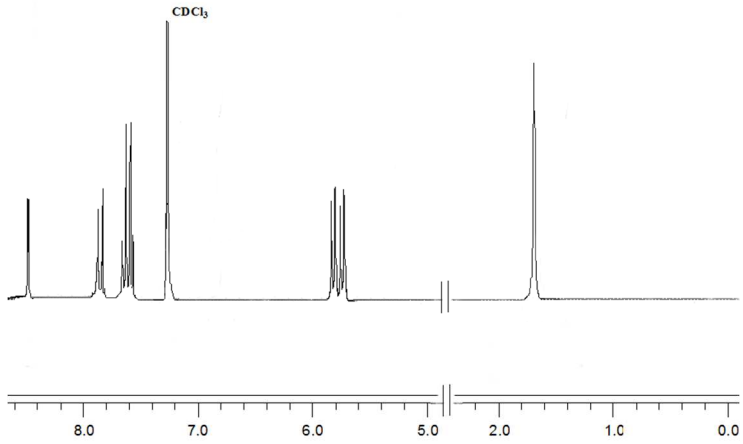
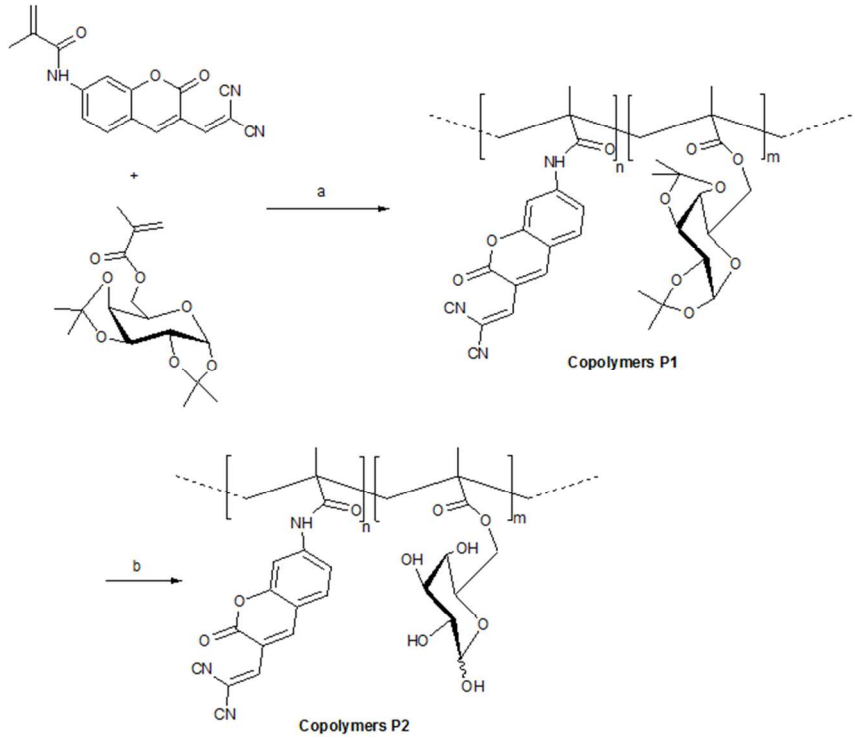


Figure 2: 1H NMR spectra of the monomer **7.**

2.2 Copolymerization of 7 with S_{1b}

The free radical copolymerization of **7** with $\mathbf{S_{1b}}$ was performed in chloroform for 8 h at 65 °C using AIBN as an initiator as reported in scheme 2. Different monomer ratios were considered. The copolymers $\mathbf{P_1}$ were obtained with good yields (60– 95%), and then, they were deprotected by using TFA solution to afford the deprotected polymers $\mathbf{P_2}$.



Scheme 2: Synthesis of the copolymers **P1** and **P2**. Reagents and conditions (a) AIBN, $CHCl_3$, 65°C, 8h (b) TFA, RT, 2h.

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The Table 1 reports the values of mass average molecular weight (Mw) and number average molecular weight (M_n) and polydispersity index (I) of copolymers **P2a-e** which are determined by size exclusion chromatography. The data indicate that *Mw* and *Mn* were found to range from 11600 to 19600 g/mol and 6400 to 8700 g/mol, respectively. With all these glycoconjugated polymers with different sugar ratio, we can compare their solubility in water and their glycidic ratio (Table 1). The water solubility is immediate with the all polymers except the polymers with glycidic ratio under 50% which are insoluble and poorly soluble, respectively, in water. Therefore we can conclude that for these polymers **P2a-e** to be soluble, a minimum glycidic ratio of 50% is required.

Table 1: Molecular weight data and water solubility for the copolymerization of 7 with S1b

copolymers	Ratio glycide/7	Mw	Mn	I	Water solubility (g/L)[a]
P2a	30/70	11600	6400	1.81	Insoluble
P2b	40/60	15100	8100	1.86	Poorly soluble
P2c	50/50	19600	8700	2.25	81
P2d	60/40	nd	nd	nd	113
P2e	80/20	nb	nd	nd	203

The chemical structures of co-polymers **P2c** were confirmed by spectroscopic methods (IR and ^{1}H NMR).

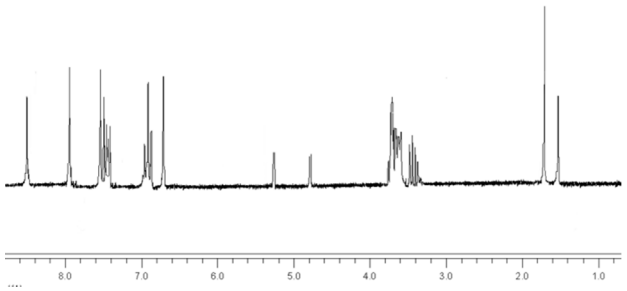

Figure 3: ^{1}H NMR spectrum of the co-polymers **P2c**

The IR spectra of the copolymers showed strong absorption bands characteristic of the carbonyls of methacrylate and cinnamate functions at about 1755 cm^{-1} and 1720 cm^{-1}, respectively. A weak absorption band at 1613 cm^{-1} was assignable to the CH=C unsaturation of cinnamate groups. A strong absorption bands characteristic of the glycidic hydroxyl groups appear at 3140 cm^{-1}. The ^{1}HNMR (figure 3) of the copolymer **P2c** shows a signals at δ= 7.94 - 6.83 ppm due to the aromatic protons. The proton of the C*H*=C unsaturation is observed at δ= 8.55 ppm. The presence of the glycidic part on the copolymers **P2c** is confirmed by the presence of the signals at 5.28-3.30 characteristic of the galactoyl moiety. The peaks at δ = 1.78 and 1.67 ppm correspond to the CH_2 and CH_3 protons of ethyl and methyl groups respectively of the co-polymers.

2.3 Fluorescence spectra titration of P2c with various halide ions

The water polymer **P2c** shows a fluorescence emission band with a maximum at **580** nm with a high fluorescence quantum yield (**0.51**) in in buffered aqueous solution (50 mM 50 HEPES, pH=7.4). Figure 4 reports the fluorescence emission spectra of the water soluble copolymer **P2c** in the presence and the absence of a different anions (CH_3COO^-, HSO_4^-, ClO_3^-, ClO_4^-, Cl^-, Br^-, $H_2PO_4^-$, $S_2O_3^{2-}$, F^-, NO_3^-, I^-, NO_2^-, SO_3^{2-}, S^{2-}, $C_2O_4^{2-}$, SO_4^{2-}, N_3^-, SCN^-, CO_3^{2-}, HCO_3^- and CN^-). As shown in Figure 3, upon addition 50 equivalents of CN^-, the fluorescence emission band with a maximum at **581** nm is blue shifted **to 500** nm with a slight decrease of the fluorescence quantum yield (**0.35**) in buffered aqueous solution. However, upon addition the other metal ions, no significant changes in the fluorescence emission spectra were observed both in buffered aqueous solution. This interesting feature revealed that the copolymer **P2c** can be used as selective fluorescent chemosensor for the cyanide anions in water for possible applications of the present system in the anion analysis in the biological mediums.

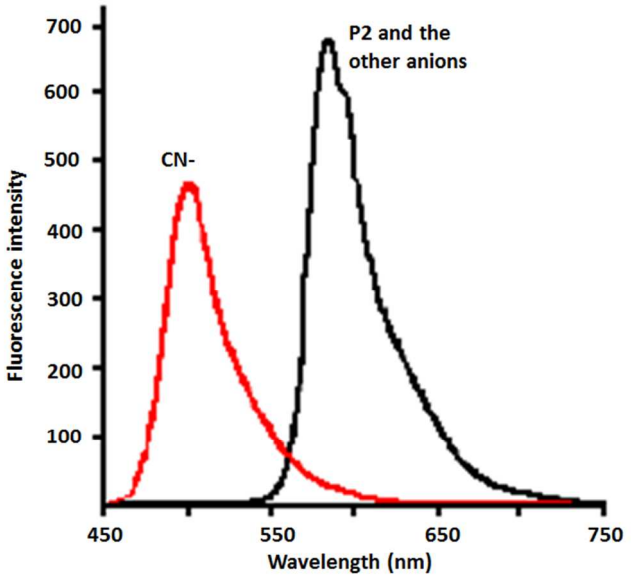

Figure 4: Fluorescence spectra (λ_{ex}= 460 nm) of **P2c** (50 µM) upon the addition of various (CH_3COO^-, HSO_4^-, ClO_3^-, ClO_4^-, Cl^-, Br^-, $H_2PO_4^-$, $S_2O_3^{2-}$, F^-, NO_3^-, I^-, NO_2^-, SO_3^{2-}, S^{2-}, $C_2O_4^{2-}$, SO_4^{2-}, N_3^-, SCN^-, CO_3^{2-}, HCO_3^{2-} and CN) in buffered aqueous solution (50 mM HEPES, pH=7.4).

In addition to the changed fluorescent behavior caused by the

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tuned D-π-A structure of fluorophore **7** in the copolymer **P2c** in the presence of the cyanide anions, the color change was another apparent phenomenon. The copolymer **P2c** contains a coumarin derivative as the fluorophore and a dicyano-vinyl group as a putative cyanide-dependent reactive subunit. First, the **P2c** was with a red color, upon addition of the cyanide anion to the buffered solution of **P2c**, The cyanide anions attack the α-position of the dicyano-vinyl group in the fluorophore **7** of the copolymer **P2c**, to generate the stabilized anionic specie of **P2c -CN**, which presented a yellow color (figure 5).

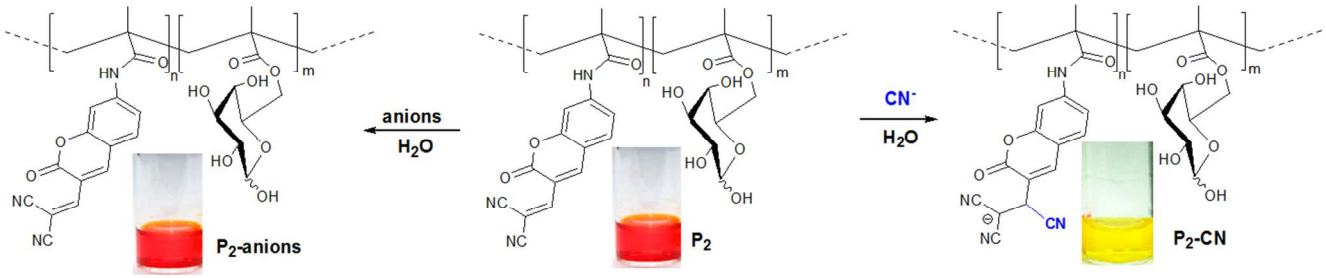

Figure 5: The colour and sensing mechanism of the copolymer **P2c** in the presence of the cyanide and other anions (CH_3COO^-, HSO_4^-, ClO_3^-, ClO_4^-, Cl^-, Br^-, $H_2PO_4^-$, $S_2O_3^{2-}$, F^-, NO_3^-, I^-, NO_2^-, SO_3^{2-}, S^{2-}, $C_2O_4^{2-}$, SO_4^{2-}, N_3^-, SCN^-, CO_3^{2-}, HCO_3^{2-}).

The fluorescence intensity of the copolymer **P2c** upon treatment with CN- (50 mM) was measured in the presence of 100 mM of the different anions (CH_3COO^-, HSO_4^-, ClO_3^-, ClO_4^-, Cl^-, Br^-, $H_2PO_4^-$, $S_2O_3^{2-}$, F^-, NO_3^-, I^-, NO_2^-, SO_3^{2-}, S^{2-}, $C_2O_4^{2-}$, SO_4^{2-}, N_3^-, SCN^-, CO_3^{2-}, HCO_3^- and CN^-). The presence of background anions did not cause any significant quenching ratio (F/F_0) change of P2 as reported in figure 6.

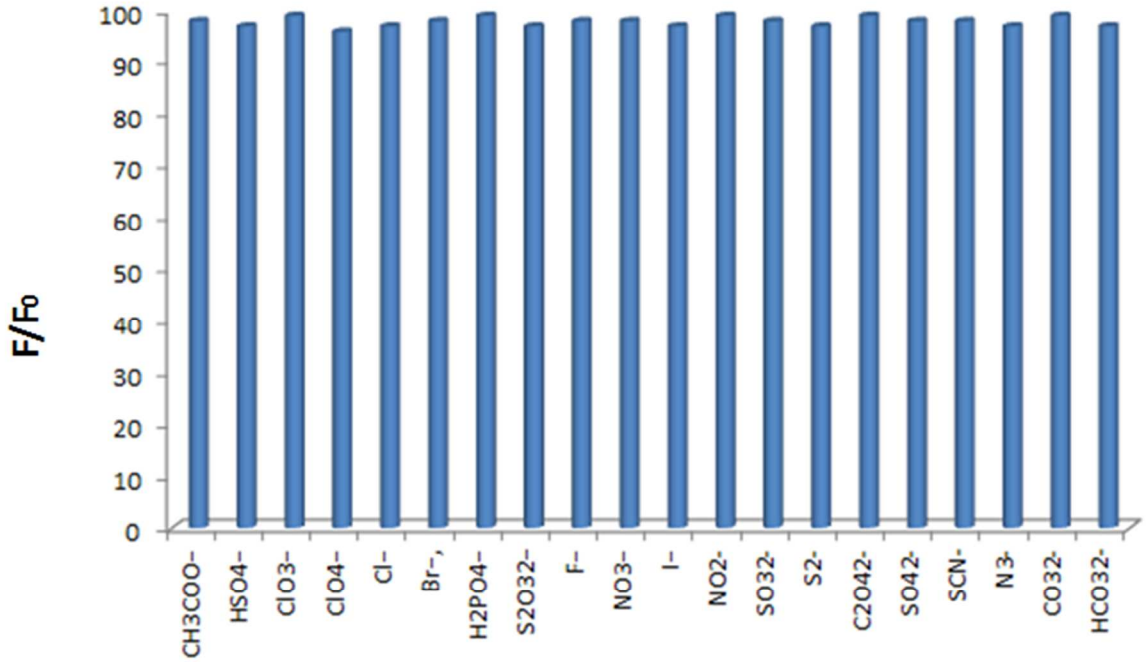

Figure 6: Quench ratio (F/F_0) of the fluorescence intensity of **P2c** (50 mM) in buffered aqueous solution (50 mM HEPES, pH=7.4) upon the addition of 50 mM of CN^- in the presence of 100 mM of background anions (CH_3COO^-, HSO_4^-, ClO_3^-, ClO_4^-, Cl^-, Br^-, $H_2PO_4^-$, $S_2O_3^{2-}$, F^-, NO_3^-, I^-, NO_2^-, SO_3^{2-}, S^{2-}, $C_2O_4^{2-}$, SO_4^{2-}, N_3^-, SCN^- CO_3^{2-}, HCO_3^{2-}).

The results reported in the figure 6 showed that the **P2c** could selectively detect CN^- in the presence of other anions such as (CH_3COO^-, HSO_4^-, ClO_3^-, ClO_4^-, Cl^-, Br^-, $H_2PO_4^-$, $S_2O_3^{2-}$, F^-, NO_3^-, I^-, NO_2^-, SO_3^{2-}, S^{2-}, $C_2O_4^{2-}$, SO_4^{2-}, N_3^-, SCN^- CO_3^{2-}, HCO_3^{2-} in water**.**

2.4 Detection limit calculation

The detection limit[60] of chemosensor **P2c** as a cyanide fluorescent probe was evaluated from the plot of fluorescence intensity as a function of the CN^- concentration as reported in Figure 6. From a plot for the linear region, the detection limit[60] of cyanide with **P2c** was found to be 1.17 μmol L^{-1} (Figure 7) which is comparable with detection limits of the chemosensors [46-53] developed in our laboratory or in the literature.[20-33] According to the World Health Organization (WHO), cyanide concentrations

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lower than 1.9 µmol L^{-1} are acceptable in drinking water,[10] which meant that the water soluble polymer **P2c** based fluorescent method is sensitive enough to monitor cyanide concentration in drinking water.

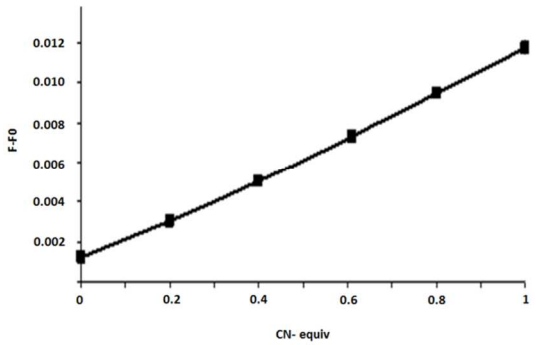

Figure 7: Calibration curve of **P2c –CN**$^-$ in HEPES (pH=7.4). The excitation wavelength was 460 nm. The concentration of the chemosensor $\mathbf{P_2}$ was 50 μM

3. Conclusions

A novel water soluble polymer **P2c** for the cyanide anions chemosensing was prepared and characterised. The chemosensor **P2c** shows a remarkably high ability to detect selectively the cyanide anions with a visible colorimetric and fluorometric changes in biological solution. The detection limit for the CN^- was calculated to be 1.17 µmol L^{-1} which meant that the water soluble polymer **P2c** based fluorescent method is sensitive enough to monitor cyanide concentration in drinking water.

4. Experimental

4.1 Materials

All chemicals were reagent grade (Aldrich Chemical Co.) and were used as purchased without further purification. Thin layer chromatography (TLC) analysis was performed using Fluka aluminium foils coated with 25 mm particle size silica gel matrix F254. TLC development involved either UV (254 and 366 nm) or visible light inspection, followed by either treatment with an acid solution of p-anisaldehyde or a basic solution of $KMnO_4$ and heating. Flash column chromatography was performed on Merck silica gel 60 (particle size 0.040 - 0.063 nm, 230 – 400 mesh ASTM) according to the procedure of Still.[63] Uv-visible spectra were recorded on a Cary-4000 Varian spectrophotometer, using either 0.1 or 1 cm quartz cuvettes. Infra-red spectra were recorded in a KBr disk on a Perkin Elmer-Spectrum BX FTIR system. Absorptions are quoted in wavenumbers (cm-1). ^{1}H and ^{13}C NMR spectra were recorded at 200 MHz 1H (50.0 MHz ^{13}C) on a Varian Gemini spectrometer. Spin resonances are reported as chemical shifts (d) in parts per million (ppm) and referenced to the residual peak as an internal standard of the solvent employed, as follow: $CDCl_3$ 7.27 ppm (^{1}H NMR), 77 ppm (^{13}C NMR, central band), DMSOd6 2.50 ppm (1H NMR, central band), 39.5 ppm (13C NMR, central band). Spin multiplicity is showed by s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, br=broad. Coupling constants *J* are reported in Hertz. Mass spectra were recorded on a ThermoScientific LCQ-Fleet mass spectrometer under electrospray ionisation (ESI, +c or −c technique). High Resolution Mass Spectra (HRMS) were recorded on aLTPOrbitrap mass spectrometer from Thermo Electron Corporation under ESI (+c) technique. Mass spectrometric analysis is quoted in the m/z form. Elemental analyses were recorded on a Perkin Elmer 240 C Elemental Analyzer. The derivatives $\mathbf{S_{1a}}$ was prepared following our previous procedure [49].

4.2 The chemosensor 7 concentration determination

The initial concentration of the fluorophore 7 C_0 and its concentration C_1 after the copolymerisation (after separation from the copolymerisation solution) were determined by using HPLC (C18 column, reversed-phase). The quantitative determination of the fluorophore was carried out by UV detector settled at 494 nm. The concentration of the fluorophore 7 in the obtained copolymers is C_0 - C_1.

4.3 Intermediates and monomers

4.3.1 Synthesis of 7-nitro-2H-chromen-2-one (3)

2-hydroxy-4-nitrobenzaldehyde **1** (1.00 g, 5.95 mmol), diethylmalonate (2) (0.96 g, 5.95 mmol) and $Na_2CaP_2O_7$ catalyst (0.2 g) were combined in 10 mL of eco-compatible EtOH/water: 95/5 and stirred at room temperature for 20 min. Then, the catalyst was removed by filtration and washed with ethyl acetate. After concentration of the filtrate, the residue was purified by re-crystallization using EtOH leading to 7-nitro-2H-chromen-2-one **3** (1.07 g, 94%) as yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ= 8.16-8.10 (m, 3H), 7.93 (d, 1H), 6.29 (d, 1H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ= 161.3, 154.7, 147.4, 143.5, 129.4, 126.3, 120.1, 113.0, 112.3 ppm. MS (ESI): m/z = 192.21 [M + 1] +. $C_9H_5NO_4$ (191.02): C, 56.55; H, 2.64; N, 7.33 found, C, 56.63; H, 2.77; N, 7.42.

4.3.2 Synthesis of 7-nitro-2-oxo-2H-chromene-3-carbaldehyde (4)

Fresh distilled DMF (2 mL) was added dropwise to $POCl_3$ (2 mL) at 50 °C with N_2 atmosphere and stirred for 30 minutes to yield a red solution. This solution was combined with a portion of 3 (1.00 g, 5.24 mmol, dissolved in 10 mL DMF) to yield a scarlet suspension. The mixture was stirred at 60 °C overnight and then poured into 70 mL of ice water. NaOH solution 2M was added to adjust the pH of the mixture to yield large amount of precipitate. The crude product was filtered, thoroughly washed with water, dried and recrystallized in absolute ethanol to give 3 (0.80 g, 69%) as yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ= 9.98 (s, 1H), 8.38 (s, 1H), 8.17-8.11 (m, 3H) ppm. ^{13}C NMR (50 MHz, CDCl3) δ= 187.5, 159.5, 154.6, 147.5, 147.1, 134.7, 129.3, 124.4, 120.4, 112.8 ppm. MS (ESI): m/z = 220.17 [M + 1] +. $C_{10}H_5NO_5$ (219.02): C, 54.81; H, 2.30; N, 6.39found, C, 54.88; H, 2.39; N, 6.46.

4.3.3 Synthesis of 2-((7-nitro-2-oxo-2H-chromen-3-yl) methylene) malononitrile (5)

7-nitro-2-oxo-2H-chromene-3-carbaldehyde **4** (1.00 g, 4.54 mmol), Malononitrile (0.73 g, 4.54 mmol) and $Na_2CaP_2O_7$as catalyst (0.2 g) were combined in 15 mL of eco-compatible EtOH/water: 95/5 and stirred at room temperature for 15 min. Then, the catalyst was removed by filtration and washed with

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ethyl acetate. After concentration of the filtrate, the residue was purified by re-crystallization using EtOH leading to **5** (1.12 g, 95%) as yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ= 8.16-8.11 (m, 3H), 7.77 (s, 1H), 7.53 (s, 1H) ppm. ^{13}C NMR (50 MHz, CDCl3) δ= 165.5, 161.7, 154.2, 147.6, 138.6, 129.3, 124.3, 120.2, 119.6, 116.4, 112.7, 105.5 ppm. MS (ESI): m/z = 268.35 [M + 1] +. $C_{13}H_5N_3O_4$ (267.03): C, 58.44; H, 1.89; N, 15.73 found, C, 58.52; H, 1.94; N, 15.80.

4.3.4 Synthesis of 2- ((7 – amino – 2 – oxo - 2H – chromen – 3 -yl) methylene) malononitrile (6)

To a solution of **5** (1. 00g, 3.74 mol) in methanol (10 ml), $SnCl_2$ (2 mol)] was added and the resulting mixture was stirred under reflux. After the completion of the reaction (monitored by TLC), the reaction mixture was filtered through celite. The filtrate was evaporated under vacuum and the residue was taken into chloroform, washed twice with 80% saturated brine solution and finally with water. The organic layer was dried over anhydrous sodium sulphate and evaporation of the organic layer was followed by purification either by column chromatography (AcOEt/PE: 10/3, Rf=0.47) to yield **6** in 84/ as yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ= 7.75 (s, 1H), 7.54 (s, 1H), 7.32 (d, 1H), 6.59 (d, 1H), 6.31 (s, 1H), 6.22 (s, 2H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) δ= 165.4, 161.5, 156.3, 152.3, 138.7, 129.3, 119.5, 116.6, 110.5, 108.4, 105.4, 100.9 ppm. MS (ESI): m/z = 238.45 [M + 1] +. $C_{13}H_7N_3O_2$ (237.03): C, 65.82; H, 2.97; N, 17.71 found, C, 65.92; H, 3.11; N, 17.85.

4.3.5 Synthesis of N-(3-(2,2-dicyanovinyl)-2-oxo-2H-chromen-7-yl) methacrylamide (7)

To a solution of the dye **6** (1.00g, 4.21 mol) in 15 mL of THF, methacrylic chloride (0.44g, 4.21 mol) was added and the mixture was stirred at room temperature for 1 h. TLC showed the formation of one major spot at (Rf. 0.53, dichloromethane / methanol: 10:0.20). The reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in chloroform (20mL) and washed with solution of HCl 5% (20mL) and water (3 x 20mL). The organic solution was dried over Na_2SO_4 and filtered, the filtrate was concentrated under the reduce pressure and the residue was purified by Flash chromatography (dichloromethane/methanol: 10:0.20) to afford **7** in 71%) as yield. ^{1}H NMR (200 MHz, $CDCl_3$) δ= 8.51 (s, 1H), 7.84 (s, 1H), 7.78 (s, 1H), 7.58-7.55 (m, 3H), 5.85-5.60 (2d, 2H, CH_2=), 1.77 (s, 3H), ppm. ^{13}C NMR (50 MHz, CDCl3) δ= 165.6, 163.4, 161.4, 156.1, 141.3, 138.8, 135.2, 128.5, 119.7, 118.5, 118.1, 116.8, 113.6, 111.7, 105.6, 40.2, 21.9 ppm. MS (ESI): m/z = 306.42 [M + 1] +. $C_{17}H_{11}N_3O_3$ (305.08): C, 66.88; H, 3.63; N, 13.76 found, C, 66.93; H, 3.69; N, 13.82.

4.3.6 6-O-(methyl methacrylate) -1,2:3,4-di-O-isopropylidene-α-D-galactopyranose (S_{1b})

To a solution of S_{1a} (1.00g, 3.84 mmol) in 15 mL of THF, methacrylic chloride (0.41g, 3.84 mmol) was added and the mixture was stirred at room temperature for 1h. TLC showed the formation of one major spot at (Rf. 0.49, AcOEt/PE: 3:2). The reaction mixture was evaporated to dryness under reduced pressure. The residue was dissolved in chloroform (20 mL) and washed with solution of HCl 5% (20mL) and water (3 x 20mL). The organic solution was dried over Na_2SO_4 and filtered, the filtrate was concentrated under the reduce pressure and the residue was purified by Flash chromatography (AcOEt/PE: 3:2) to afford **S1b** in 86%) as yield. ^{1}H NMR (200 MHz, $CDCl_3$): see table 2 for the glycidic part and δ = 6.14 and 5.90 (2d, 2H, CH_2=), 2.08 (s, 3H), 1.51, 1.45, 1.34, 1.33 [4s, each 3 H, 2 x C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 167.3, 136.8, 125.2, 109.1, 108.3 [2 x $C(CH_3)_2$], 95.7, 70.5, 70.1, 69.8, 65.4, 63.2, 25.4, 25.3, 24.4, 23.9 [2 x $C(CH_3)_2$], 18.7 ppm. MS (ESI): m/z = 329.36 [M + 1] $^+$. $C_{16}H_{24}O_7$ (328.15): C, 58.52; H, 7.37 found, C, 58.63; H, 7.44.

Table 2: ^{1}H NMR spectroscopic data (δ, ppm; J, Hz) in $CDCl_3$ of the glycide portion for protected 6-O-D-galactoyl (**S1b**) derivatives.

1-H	2-H	3-H	4-H	5-H	6a-H	6b-H
5.54	4.34	4.63	4.30	4.01	4.18	4.21
$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
5.0	2.5	7.9	1.8	7.4	4.8	10.2

4.3.7 Synthesis of the polymers P1a-b

Five copolymerization experiments were performed using **7** and S_{1b} as co-monomers. Various monomer molar ratios S_{1b}/**7** (a- 30/70, b- 40/60, c- 50/50, d- 70/30 and e- 80/20) were considered. Appropriate amounts of monomers, initiator (AIBN, 1% compared to 7), and chloroform (5 ml) were introduced in a round-bottom flask fitted with a condenser. The mixture was placed under nitrogen atmosphere, and then stirred at 65 °C for 8 h. The polymers formed were concentrated by evaporation and purified by precipitation with methanol (5 mL) and diethyl ether (100 mL). After drying in vacuum, the polymers **P1a-e** were obtained as a red solid. Yield: 65–90%. ^{1}H NMR (200 MHz, $DMSOd_6$) δ= 8.57 (s, -C*H*=C-), 8.11 (s, N*H*), 7.96 (s, Ar-*H*), 7.58-7.54 (m, Ar-*H*), 7.01-6.89 (m, Ar-*H*), 6.71 (s, Ar-*H*), 5.57 (m, gly-H), 4.61 (m, gly-H), 4.37-4.08 (m, gly-H), 1.79 (s, CH_2), 1.69 (s, CH_3), 1.52, 1.45, 1.32, 1.31 [4s, each 3 H, 2 x C(CH_3)$_2$] ppm. ^{13}C NMR (50 MHz, $DMSOd_6$) δ= 176.5, 175.8 (CO), 165.6 (=CH), 161.6 (Ar C), 156.4 (Ar C), 139.2 (Ar =CH). 138.9 (Ar C), 128.2 (Ar C) 119.5 (=C−CH=), 109.2, 108.6 [2 x $C(CH_3)_2$], 118.4 (Ar C), 113.4 (Ar C), 111.7 (Ar C), 105.7 (N≡C−C), 95.4, 70.6, 70.3, 69.5, 65.6, 63.0, 43.6 (C−CH_3), 38.7, 37.5 (CH_2), 25.5, 25.2, 24.6, 23.6 [2 x C(CH_3)$_2$] 22.5, 22.1 (CH_3), ppm.

4.3.8 Synthesis of the polymers P2a-e

A solution of **P1a-b** (1.00 g) in 90% aqueous TFA (15 mL) was stirred at room temperature for 3 h. The violet solution was repeatedly co-evaporated with toluene (5 x 25 mL) at reduced pressure to give the final product **P2a-e** (0.78 g) as a red powder consisting of a mixture of α- and β-pyranosic anomers in a ratio of 50:50, calculated on the basis of the relative *C-1* signal intensities. ^{1}H NMR (200 MHz, D_2O) δ= 8.55 (s, -C*H*=C-), 8.13 (s, N*H*), 7.94 (s, Ar-*H*), 7.57-7.52 (m, Ar-*H*), 7.00-6.85 (m, Ar-*H*), 6.72 (s, Ar-*H*), 5.28-5.20 (d, gly-H), 4.78 (d, gly-H), 3.83-3.61 (m, gly-H), 3.47-3.31 (m, gly-H), 1.78 (s, CH_2), 1.67 (s, CH_3) ppm. ^{13}C NMR (50 MHz, D_2O) see Table 3 for the glycidic part and δ= 176.4, 175.6 (CO), 165.5 (=CH), 161.3 (Ar C), 156.7 (Ar C), 139.4 (Ar =CH). 138.6 (Ar C), 128.5 (Ar C) 119.4 (=C−CH=), 118.2

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(Ar C), 113.3 (Ar C), 111.9 (Ar C), 105.4 (N≡C−C), 43.5 (C−CH_3), 38.6, 37.2 (CH_2), 22.9, 22.3 (CH_3) ppm.

Table 3. ^{13}C NMR spectroscopic data (δ, ppm) for the glycide portions for deprotected 6-*O*-D-galactoyl derivatives.

	solvent	C-1	C-2	C-3	C-4	C-5	C-6
P2-αp	D_2O	92.4	72.2	81.9	72.1	70.1	61.2
P2-βp	D_2O	96.9	76.7	85.2	74.6	69.8	61.1

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