



Influence of pH, ionic strength and natural organic matter concentration on a MIP-Fluorescent sensor for the quantification of DNT in water



Jingjing Dai, Maria Fidalgo de Cortalezzi*

Department of Civil and Environmental Engineering, University of Missouri, E2509 Lafferre Hall, Columbia, MO, 65211, USA

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ABSTRACT

The effect of sample water chemistry on a carbon dot labeled molecularly imprinted polymer (AC-MIP) sensor for the detection of 2,4-dinitrotoluene (DNT) was investigated. Hydrogel MIP films were fabricated and tested in DNT solutions in various matrices, representative of natural water conditions, to assess applicability of the sensors to real water samples. The effect of pH, natural organic matter (NOM), ionic strength and cation type on the swelling of the hydrogel and fluorescence quenching was investigated. An increase in ionic strength from 1 mM to 100 mM produced a quenching amount of MIPs decreased of about 19% and 30% with NaCl and CaCl₂ respectively. In the range of pH tested, from 4 to 9, quenching was higher at basic environment for both MIPs and non-imprinted polymers (NIPs) due to increased hydrogel swelling. NOM contributed to the background quenching, but the effect could be addressed by an adjusted calibration equation. In both lake and tap water, DNT concentrations read by the sensors were close to the values measured by HPLC, within 72%–105% of true values. The AC-MIP films fabricated in this work are promising materials for the detection of water contamination in the field and the quantitative analysis of DNT concentration.

1. Introduction

Molecular Imprinted Polymer (MIP) based sensors had been successfully applied to the quantitative detection of specific contaminants. In order to obtain a useful sensor device, different transducer mechanisms have been combined with MIPs, based on optical, chemical or electrochemical properties. Among them, optical MIP-based molecular recognition sensors have been developed due to the covalent linkage of MIPs to optical transducers. The MIP binding efficiency, recognition processes and selectivity have been intensively studied by optical means due to the general high sensitivity and simplicity of the utilization of optical techniques [1]. The sensing properties can also be characterized by parameters such as linearity, limit of detection, sensitivity, selectivity, reproducibility, accuracy, robustness, response time and lifetime [2]. However, the effects of sample characteristics commonly encountered in natural waters (i.e. variations of pH, ionic strength, natural organic matter content) on the sensing performance of optical MIPs are not fully understood, which is a limitation for environmental applications. MIPs have been reported which respond to stimuli such as change in temperature [3], pH [4], and ionic strength [5]. Interferences, either with the imprinted molecule or transducer mechanism, are another concern because they can affect the recognition ability and sensing response of MIPs. Potential interferences in natural waters are dissolved organic

matter, dissolved salts, inorganic colloidal particles, and microorganisms. Given the fact that most efforts have been devoted to the synthesis of MIPs as solid phase extraction (SPE) media, research in this area has been directed mostly towards the conditioning, washing and eluting stages of the process rather than the capture and sensing, or the actual mechanisms by which this interference may occur. The potential for MIP-based sensors to be used in-situ is promising if the influences of these parameters are explored and interpreted accurately.

Some researchers have reported the use of MIPs as the SPE sorbent for natural water samples [6, 7], but the direct use of MIPs and MIP-enabled devices as sensors in natural waters has not been fully explored [8, 9], especially regarding the effects of the different parameters of the water chemistry matrix. A conductometric transducer based atrazine sensor was reported for the monitoring of atrazine and structural analogs, both in contaminated and uncontaminated natural waters with a detection limit of 1 ppb [10, 11]. In another study, diphenolic Acid (DPA) and bisphenol A (BPA) were used as dummy template molecules for the capture of tetrabromobisphenol A (TBBPA). With recoveries of TBBPA in tap, river and lake water ranged from 85% to 97% and detection limit as low as 2 ppb [12].

Dinitrotoluene (DNT) is an aromatic explosive that exists as six isomers; the forms 2,4-DNT and 2,6 DNT account for more than 95% of technical grade DNT and are the ones used in the synthesis of the

* Corresponding author.

E-mail address: fidalgom@missouri.edu (M. Fidalgo de Cortalezzi).

explosive trinitrotoluene (TNT) [13, 14]. DNT is also employed in the manufacturing of polyurethanes, dyes, plastics and herbicides [15]. Due to its water solubility (300 ppm at 22 °C) and low vapor pressure (1.47×10^{-4} mmHg at 25 °C), 2,4-DNT may remain in water for a long time. US EPA has classified DNT as a probable human carcinogen and established a 1-day and 10-day health advisory of 1.0 ppm for 2,4-DNT in drinking water for a 10-kilogram (kg) child, which corresponds to a drinking water equivalent level of 0.04 ppm [15]. DNT is commonly detected in surface water, groundwater and soil at hazardous waste sites related to ammunition facilities or other industries that use it in as part of their process [14].

Fluorescence based sensors are particularly suited for field use, as they are generally sturdy and easy to measure. In this area, fluorescent nanoparticles are preferable to fluorescent polymers, as they have shown to be brighter and more stable; however, leakage of the nanomaterial into the sample may be a concern if the particles are not strongly bonded to the MIP matrix. We recently reported a novel C-dot fluorescent labeled MIP based sensor for the detection of 2,4-dinitrotoluene (DNT) in water samples in a range from 1 to 15 ppm, with a detection limit of 0.28 ppm; the fluorescent C-dots were covalently bonded to a hydrogel thin film polymer, which provided stability to the sensor even under several consecutive cycles of use and regeneration [16].

Sample components may interfere with the measurement of the analyte in two ways: by interacting or preventing interaction between the target compound and the MIP cavities, or by non-specifically adsorbing on the fluorescent nanoparticles, potentially leading to quenching and over or underestimations of the true concentration of the target. Detection of contaminants in natural waters at environmentally relevant concentrations is particularly challenging, due to the complexity and potential variability of its chemical composition. Although several MIP-based sensors have been reported in laboratory studies of controlled samples, the technology has not yet been successfully translated to realistic samples and environmental conditions. One of the hurdles to overcome is the correct interpretation of the sensor output, as interferences may potentially induce substantial deviations from initial calibration. Errors due to non-specific binding can be minimized if the sample is pretreated, for example by a phase change (aqueous to non-polar) of the analyte before measurement, or corrected if non-imprinted polymers are used alongside MIPs [17]. The objective of this study is the identification of sources and mechanisms of said interferences for a fluorescence-MIP sensor and the assessment of their effects on the sensor response, under environmentally relevant conditions.

We investigated the application of the amino functionalized carbon dot (AC-dot) labeled MIP sensor for the detection and quantification of DNT in natural water conditions. The effects of salinity, pH and dissolved organic matter on the sensor performance were analyzed. Two factors may play important roles: (1) interference with the nonspecific and specific adsorption of DNT onto the MIP, and (2) quenching by solutes other than DNT. Sample ionic strength and pH influence the surface charge and electrostatic interactions between polymer chains, which in turn change the morphological conformation of the film or swelling of the hydrogel. Additionally, acid based groups on the surface of the C-dots are responsible for variations in fluorescence intensity with pH, and dissolved organic matter may adsorb non-specifically to both polymer and nanoparticles. A methodology was established to minimize those effects and/or create an adjusted calibration for determination of DNT concentrations under different background water matrices.

2. Results and discussion

2.1. MIP film characterization

The fabricated MIP films were characterized with respect to key properties that would be altered in complex chemical environments: AC-Dots and polymer surface functional groups, and swelling behavior of the

hydrogel. The FTIR spectrum of AC-Dots revealed abundance of amino groups on the surface [16]. The surface basic groups resulted in a positively charged surface due to protonation of amino groups. The amino groups can be further protonated with decreasing pHs [18]. The quenching ability may be hindered when amino groups are well protonated and thus unable to form complexes with quenchers [19].

The degree of swelling of hydrogels at equilibrium depends on the cross-linking and charge densities of the polymer network. Polyacrylic acid can shrink or swell in response to changes in temperature, pH, ionic strength, solvent quality, electric field, etc. [20]. The swelling ratio (SR) was calculated from the expression (1)

$$SR = (m_s - m_d) / m_d, \quad (1)$$

where m_s is the mass of the swollen film at equilibrium and m_d is the mass of the freeze dried films.

The SR increased from pH = 4, 7 and 10, but not dramatically (Fig. 1). The swelling of the ionizable hydrogel was affected by pH, as the polymer became uncharged at low pH, reducing the electrostatic repulsion among chains, hydrophilicity and as a consequence, water adsorption [21]. The pKa's of the comonomers are 4.2 and 4.8, for PAA and PMAA respectively; therefore, the film was expected to be partially charged at the lowest pH tested and fully deprotonated for the other two conditions. This is consistent with the mild dependence of %SR on pH observed.

The %SR decreased with increasing ionic strength (pH = 6), and the trends were similar for both NaCl and CaCl₂ (Fig. 2). The swelling of ionic polymers is mainly due to electrostatic repulsion between charges in the polymer chain, and which will be lowered under conditions of increased electrolyte concentration, as confirmed by the experimental results. Interestingly, the type of counter ion did not show influence, as Na⁺ and Ca⁺² solutions produced comparable swelling. The behavior of the hydrogels is equivalent for same ionic strength levels rather than electrolyte concentration, providing evidence of the dominant effect of electrostatic interaction screening on swelling. This may affect the sensor and lead to less quenching in the presence of dissolved salts due to lower water adsorption.

2.2. Effect of pH

The sample pH can affect the operation of the sensor by altering the surface chemistry of the carbon dots and, as a consequence their fluorescence emission (FL), and by altering the conformation of the polymer matrix, resulting in deformation of the cavities available for capture. The FL mechanism of carbon dots is related to surface states determined in part by chemical groups, and thus, it is pH dependent [22]. An evaluation

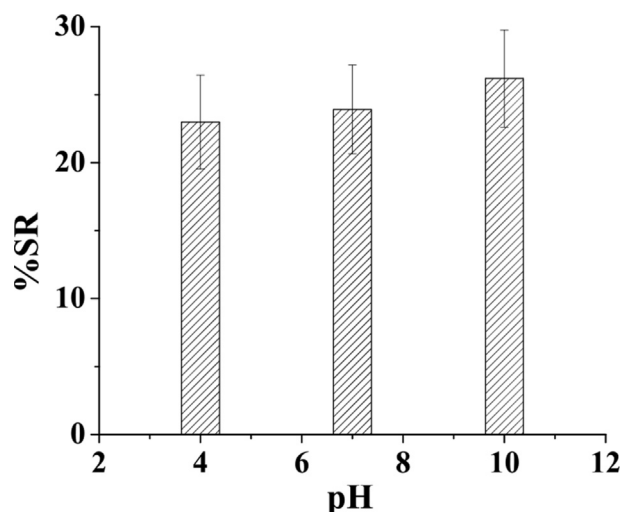


Fig. 1. Swelling ratios of PMAA films.

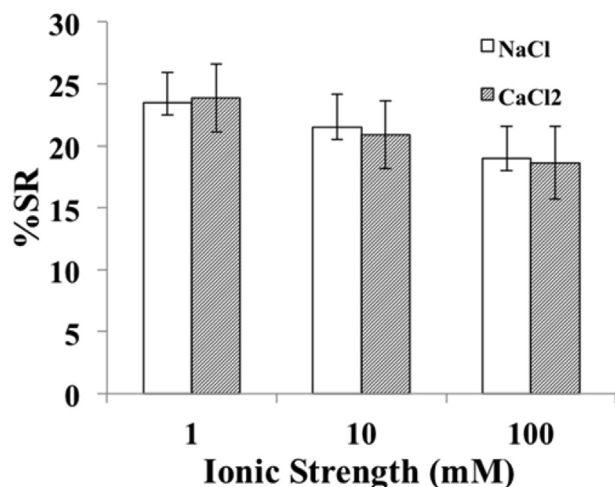


Fig. 2. Swelling Ratio% of MIP films as a function of ionic strength and salt type (pH = 6).

of such dependency showed that AC-dots (0.2 ppm in aqueous suspension) exhibited the highest FL intensity at pH 4, and decreased with increasing pH (Fig. 3). Their FL activities were strong at weakly acidic media (pH 4–6), but weaker at higher pH, especially above 10. This observation is in agreement with previous reports [19], and constitutes a limitation to the application of carbon dots as labeling materials for sensing.

Tests of the effects of pH on the MIP sensor were conducted with initial FL intensities (F_0) measured in DI water. Then, the MIP-sensors were dipped in acidic, neutral, or basic 10 ppm DNT solutions, and rinsed with DI water before measuring the FL intensities (F), again in clean water. Although the sensor initially showed different responses for the three pH levels, those were minimized after the rinsing step was introduced. In the absence of DNT, exposing the MIP films to acid or base solutions, the fluorescence intensity changed accordingly (Fig. 4). However, after the clean water rinse (pH = 6), the effects were less evident and F_0/F much closer to 1 as expected since no DNT molecules were present. This represents a significant advantage over sensing approaches involving the direct addition of C-dots to the water sample or non-covalent bonded fluorescent labels, where the inherent limitation of the pH dependence of the FL of the nanomaterials cannot be overcome.

However, a diminished selectivity of the sensor at acidic pH could not be completely avoided. The recognition capacity (RC) of the MIP is defined as the ratio of specific to non-specific adsorption, i.e. binding to

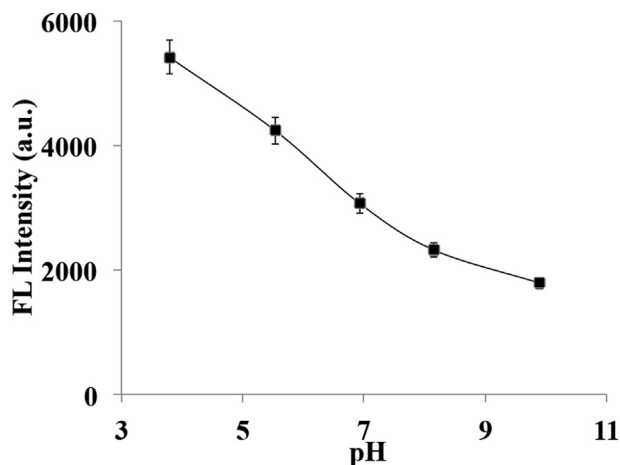


Fig. 3. Fluorescence intensity of AC-Dots suspended in aqueous solutions of variable pH.

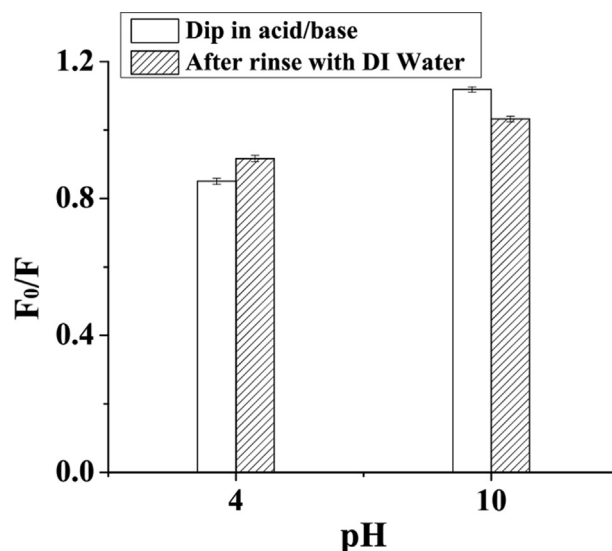


Fig. 4. FL Intensity of MIP sensors in acidic (pH = 4) and basic (pH = 10) environments and after DI water rinse, in the absence of DNT.

the imprinted molecular cavities versus adsorption to a random area of the polymer surface, and it can be considered a measurement of the selectivity of the sensor. Fig. 5 showed the F_0/F ratios for both MIPs and NIPs, after placed in contact with a 10 ppm DNT solution. RC decreased with increasing pH, as it is assumed that binding to MIPs occurs predominantly at the imprinted sites, while binding to NIPs is purely non-specific. Quenching amounts between 1.3 and 1.6 for the NIPs show the incidence of non-specific adsorption; the observed upward trend can be related to the changes induced by the sample pH to the surface of AC-Dots and the corresponding change in their FL intensity as presented in Fig. 4 after rinse with DI water, which results in an apparent lower quenching (due to increased FL) at acidic pH. F_0/F increased at a faster rate in the case of MIPs, with swollen, charged hydrogels displaying better performance. Results suggest that non-specific adsorption is fairly constant for the pH range considered, but access and binding to the imprinted cavities was favored at higher swelling conditions that resulted in more space for the diffusion of the DNT molecules between the polymer chains.

A deviation of the sample pH from the value of the measuring solution (DI water, pH = 6) would introduce a measurement error, overestimating

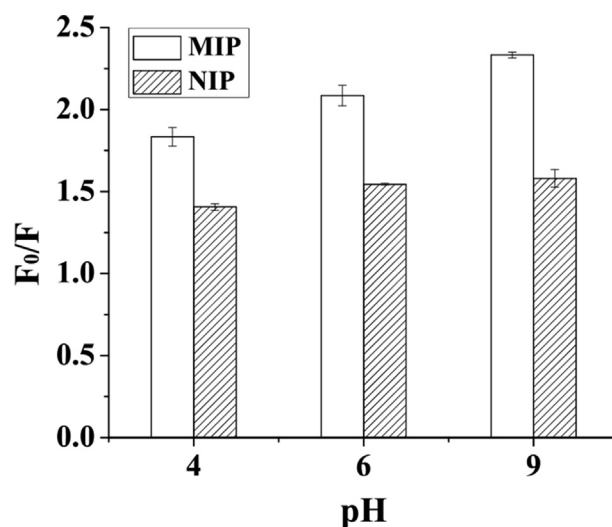


Fig. 5. Quenching of AC-Dot labeled MIPs and NIPs by DNT (10 ppm) as a function of pH.

concentration for basic pH and underestimating its value in acid solution (-8.4% at pH 4 and +3.2% at pH 10).

2.3. Effect of ionic strength and salt type

The sensor was applied to the quantification of DNT in solution with variable dissolved salts concentration, resulting in a smaller FL intensity change for both MIP and NIP films (Fig. 6). In this case, the effect of ionic strength (IS) on the FL of AC-Dots suspensions was investigated, as any possible surface effects due to charge screening in a high electrolyte concentration medium cannot be isolated from the effects on the aggregation of the nanoparticles under increased IS. The latter would dominate the FL behavior for AC-Dots in suspension but would be absent in the AC-Dot labeled sensors, making the experiment irrelevant. However, comparison FL intensities of labeled films after equilibration in different ionic strength solutions and subsequent rinsing with DI water did not showed significant differences in emission intensity and thus we focus our analysis on the effects of IS on the rebinding of DNT to the hydrogel.

As expected, NIPs showed consistently less quenching amount than MIPs (Fig. 6). With the increase in ionic strength from 1 mM to 100 mM, the quenching amount of MIPs decreased 19% and 30% in the presence of NaCl and CaCl₂, respectively. For NIPs, ionic strength from 1 mM to 100 mM, the quenching amount decreased 25% and 38% with NaCl and CaCl₂, respectively. Both cations decreased the binding of DNT; cations were reported to have a significant influence on the binding capacity following the Hofmeister series [23]. Thus, we expected to see a more profound effect for Na⁺, but larger influence for Ca²⁺ was observed. In a prior published work, we showed that calcium specifically interacts with AC-dots surface, resulting in significant alteration of its zeta potential [18], and thus can prevent further interaction with DNT molecules needed for quenching.

The diminishing gap between MIP and NIP quenching with increasing IS suggested that binding to the cavities was also affected, in agreement with the decreased swelling ratio observed with ionic strength given by both electrolytes. At pH = 6, a large fraction of the carboxylic groups in the polymer are deprotonated; the higher concentration of charged species will compress the electric double layer thickness, shortening the range of influence of the repulsive forces and producing less swelling than under DI water solutions [24]. Lower water adsorption and a deformation of the shape and size of cavities in the shrunken hydrogel contribute to the underestimation of true DNT concentrations in the samples with moderate to high ionic strength by 12%/18% and 20%/30% (NaCl/CaCl₂), respectively.

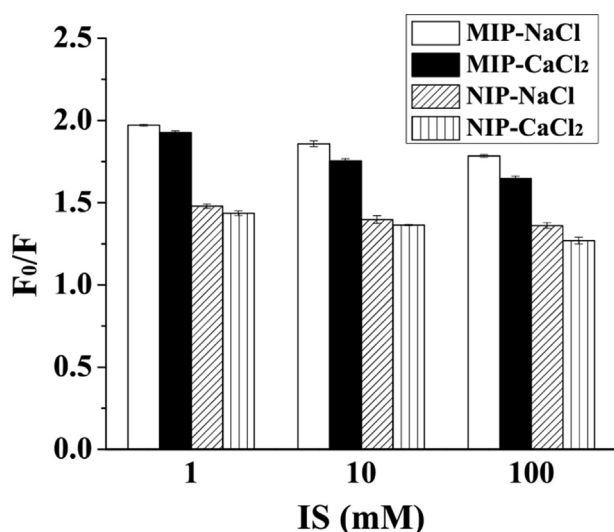


Fig. 6. Quenching by DNT (10 ppm) with different salts, pH = 6.

2.4. Effect of NOM concentration

Although their exact composition is variable, natural organic matter compounds are expected to be quenchers of AC-dots fluorescence and therefore may produce higher than true value concentrations when the sensor is used in natural waters [25]. In order to investigate this phenomenon, the quenching effect of Suwanee River Natural Organic Matter (SRNOM) was first determined in the absence of DNT, in a range of concentrations plausible to be encountered in environmental samples. Fig. 7 illustrated the decreased fluorescence intensity in the presence of variable concentration of SRNOM from a suspension of 0.2 ppm AC-dots and from the MIP films. The fitting of the experimental data points in the Stern-Volmer plot showed a linear relationship between F₀/F and SRNOM concentrations for both AC-Dots and MIP systems, (r² = 0.9991 and 0.9995).

Consequently, the quenching amount increased when NOM existed in the DNT solutions (Fig. 8) with respect to the pure water DNT solution. The rate of quenching amount increase with DNT concentration (slope of F₀/F vs. DNT concentration curve) for MIPs (0.0054) was very similar to that corresponding to the NIPs (0.0052), which implies that the rise was mainly related to nonspecific adsorption and not to interactions between the SRNOM and the imprinting cavities, such as occupying or blocking DNT access to the sites. SRNOM in samples produced a decrease in the initial intensity of the sensor, potentially impacting the limit of detection. Even in the presence of SRNOM background quenching level, the concentration of DNT could still be accurately obtained by subtracting the NOM quenching amount from the raw sensor reading.

A linear relationship was obtained for the sensor quenching due to DNT in the absence of NOM, and described by the following equation [16]:

$$F_0/F = 0.117 \times C_{DNT} + 0.98551, \quad (2)$$

The NOM content of the sample can be estimated from the NIP reading (Fig. 9) linear fit as shown by the following equation:

$$F_0/F = 0.0054 \times C_{NOM} + 1, \quad (3)$$

The NOM adjusted calibration curve for the sensor can be obtained combining Eqs. (2) and (3), resulting in Eq. (4).

$$F_0/F = 0.117 \times C_{DNT} + 0.0054 \times C_{NOM} + 1, \quad (4)$$

Applying the modified equation to the 10 ppm DNT test solutions in the presence of NOM, values of 10.2, 10.6 and 10.3 ppm, were obtained by the sensors at SRNOM concentrations 1, 10, 50 ppm respectively,

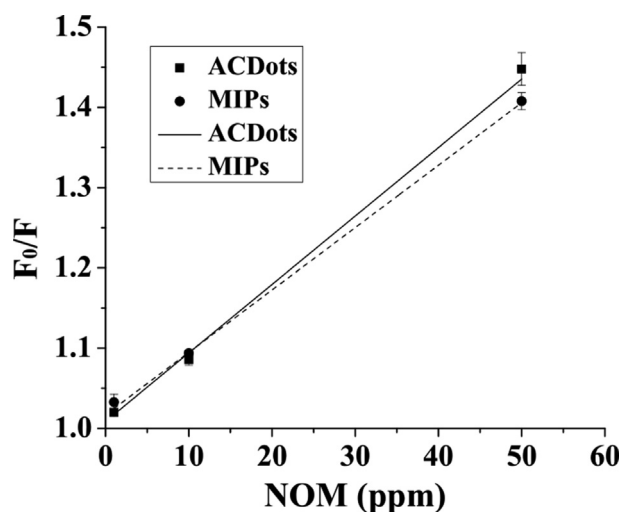


Fig. 7. Quenching of AC-Dots and AC-Dots labeled MIPs by variable concentrations of SRNOM, in absence of DNT (pH = 6).

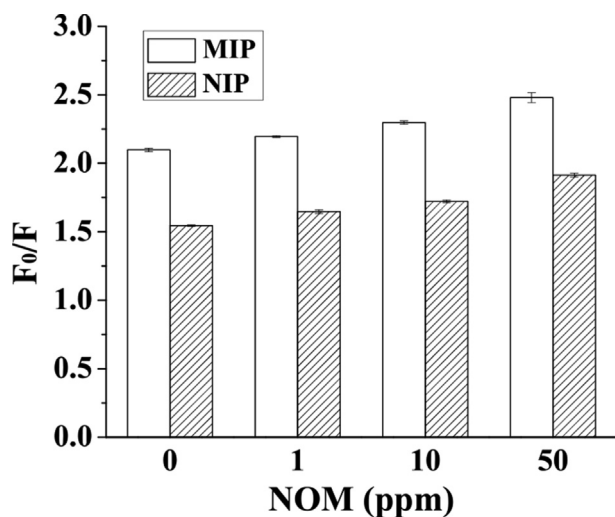


Fig. 8. Quenching by DNT (10 ppm) with Suwannee River NOM, pH = 6.

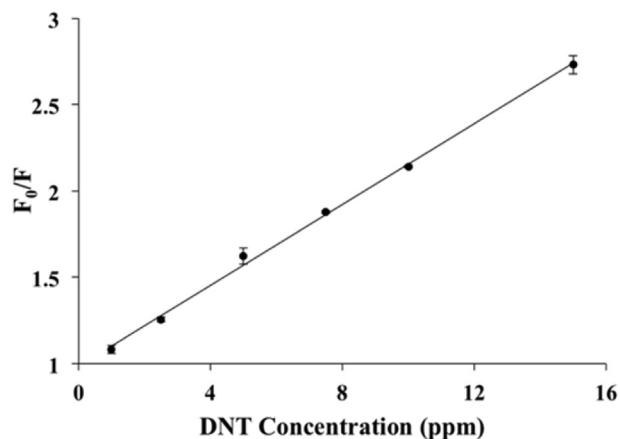


Fig. 9. Stern-Volmer plot of AC-MIPs with an increasing concentration of DNT, pH = 6.

proving the feasibility of abating NOM interference based on this method.

Sorption/complexation of organic and metal ions to humic substances is common in natural waters [26]. Therefore, it is important to note that MIPs equilibrate with free species in solution and therefore the measurement may result in underestimation of the total concentration of species if they bind to significant level to dissolved organic matter in the samples.

2.5. Application to real water samples

Real natural water samples are complex solutions, where pH, dissolved solids and natural organic compounds are probable challenging components for the sensor.

To further test its applicability in the environmental field, the AC-MIP films were used for the determination of DNT concentrations in two real water samples: Stephens Lake water and tap water from our laboratory at the University of Missouri. The collected samples were spiked with DNT and its concentration measured by the newly fabricated sensor and compared to the results from the HPLC analysis.

The experiments were conducted at different DNT concentrations, and the corresponding analysis results are listed in Table 1. DNT concentrations were calculated using Eq. (4).

Lake water was slightly basic while tap water had a neutral pH. The IS

of both lake and tap water was close to 10 mM, which based on the above reported results, was expected to have minor effects on the sensor performance. For the lake, the basic pH may result in overestimation of DNT concentrations, while medium IS could do the opposite. As expected, the TOC level of the lake water was higher than tap water due to the existence of dissolved NOM. Taking NOM in account and assuming $NOM = TOC - TOC_{dnt}$, adjusted readings were obtained. The readings by sensors in tap water were closer to the HPLC analysis than in lake water. The sensor consistently underestimated the DNT concentration in the lake sample; in addition to the measured higher TOC content, lake water may have contributions of other unknown pollutants and particulate matter that can further interfere with the sensor detection. Stephen's lake is located in an urban environment and susceptible to anthropogenic impact as well as natural organic matter inflows, especially after rainfall events. The matrix complexity results in a higher chance to block the cavities or the surfaces of sensor films. Structurally similar compounds may bind to the imprinted cavities, resulting in errors. For example, water samples with equal molar concentration of DNT and phenol, resulted in a 12% underestimation of the DNT concentration [16]. Due to its similar size and functionality, phenol was adsorbed on some of the imprinted cavities but its inability to quench the fluorescent signal resulted in a diminished reading. Alternatively, a sample of DNT and dinitrophenol at equal molar concentrations resulted in a 22% increase in quenching amount, as both compounds have two nitro groups with quenching ability. Although the errors induced by the presence of the analogs are significant, those were produced by very high concentrations of extremely similar compounds; however, organic compounds in general are mostly expected to induce interference by non-specific adsorption, weaker and more reversible than the binding to the imprinted cavities, and only if they have the ability to quench the C-dot fluorescence. In another experiment, 4-nitrophenol was tested instead of dinitrophenol in a solution at equal molar concentration as DNT; the overestimation of the target concentration dropped to less than half its original level due to the loss of the second nitro group of the analog [16].

Still, the values in both tap and lakes were reasonably close to the HPLC measured DNT concentrations. The analytical method EPA-RCA 8330B, HPLC using an ultraviolet detector, reports a multi-laboratory variance of 7 % RSD for 2,4-DNT water samples in the order of 0.1 ppm [27]. Given the low cost and simplicity of use of the MIP sensor, the measurements obtained can be considered satisfactory. The results are promissory, showing good recognition ability in challenging, unknown natural water samples.

3. Conclusions

The influence of sample pH, ionic strength and NOM content on the rebinding and fluorescence quenching process are concerns regarding the applicability of the AC-Dot labeled MIP sensor to environmental sensing. Surface chemistry of carbon dots can be affected by pH, resulting in fluorescence emission change, and higher swelling of the polymer favors the rebinding of DNT. These effects resulted in higher quenching at basic environments, which translates in slightly higher readings than real DNT concentrations. The pH effects can be partially averted if the sensors are thoroughly rinsed with clean water before the fluorescence measurement, as this process does not result in loss of bound DNT or C-dot particles. The rinsing is intended to neutralize pH and record sensor fluorescence at the calibration curve conditions of circumneutral pH. While C-dot surface group degree of deprotonation will equilibrate with rinsing water pH, the binding of DNT molecules will not be reversed. On the other hand, with increasing ionic strength, the sensor provided lower than true DNT concentrations due to lower swelling and obstruction of AC-Dot - DNT interactions in the presence of Ca^{+2} . NOM acted as a nonspecific quencher, increasing the background "noise" level and negatively impacting detection limit. However, NOM quenching showed to be predominantly non-specific in nature and therefore independent of DNT concentration. Since NOM quenching also obeys the Stern-Volmer

Table 1
Detection of DNT in natural water samples.

Sample	Sensor measured DNT concentration (ppm)	Stdev	Error (%)	HPLC measured DNT concentration (ppm)	pH	IS (mM)	TOC (ppm)
Lake	5.53	0.54	12.0	6.28	8.5	7.5	4.9
	5.77	0.46	9.7	7.99			
	12.32	1.86	18.4	14.67			
Tap	4.02	0.77	20.1	3.83	7.2	13.8	Nondetectable
	5.53	0.54	12.0	6.28			
	5.77	0.46	9.7	7.99			

equation [28], a modified expression including NOM concentration as a parameter was developed. For real water samples, the sensor was able to provide an accurate estimation of DNT concentration in tap water, with a weaker performance for the more complex lake water sample. We conclude that AC-dots labeled DNT-MIP films fabricated in this work are promising materials for in situ detection of DNT contamination in the environment.

4. Materials and methods

4.1. Materials

Chemicals were purchased from Sigma-Aldrich and used without further purification; tetraethoxysilane (TEOS) (98%), ammonia solution (25% in water), ethanol (200 proof, ACS reagent, 99.5%), acrylic acid (AA) (99%), methyl acrylate (MA) (99%), ethylene glycol dimethacrylate (EGDMA) (98%), 2,2'-azobisisobutyronitrile (AIBN) (98%), 2,4-dinitrotoluene (DNT) (97%), phenol, 4-nitrophenol (4-NP) (99%), and dinitrophenol (DNP) (98%), hydrofluoric acid (HF) (48%), citric acid (CA) (99.5%), branched polyethylenimine (BPEI), N-hydroxysuccinimide (NHS), and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC).

NaOH and HCl (Fisher Scientific) were used for pH adjustment; ionic strength of the samples was controlled by addition of NaCl (reagent grade, Acros Organics) or CaCl₂ (reagent grade, J.T.Baker). Suwannee River NOM (SRNOM) was used as a model of natural organic matter and purchased from International Humic Substances Society (St. Paul, MN, USA).

4.2. Synthesis of MIPs and fluorescent labeling

The fluorescence labeled MIP films were prepared as described in our previous work [16]. Non-imprinted polymer films (NIP) films were synthesized following a similar procedure without adding DNT and played as control for the MIP sensors in the tests. The porosity of the MIP films was created using colloidal crystals made of silica particles (~200 nm diameter) as templates followed by etching of the particles. Stöber method [29] was applied by mixing TEOS (8.3 mL), ethanol (200 mL) and ammonia (18.3 mL). The particles were purified by several consecutive washing cycles with ethanol and DI water. Colloidal crystals were fabricated by vertical self-assembly on a glass substrate as described elsewhere [30]. In this procedure, a cleaned glass slide was vertically placed into a flask containing a 0.5% volume fraction suspension of silica particles in ethanol until the formation of colloidal crystals on the slide. One end of glass assembly was put in contact with the polymerization mixture that rose by capillary forces filling the void spaces within the colloidal crystal. Polymerization solutions were prepared mixing 0.4 mL (4.4 mmol) AA and 0.4 mL (5.8 mmol) MA as the functional monomer (with the molar ratio of 3:4), 0.55 mL (2.9 mmol) EGDMA as the cross-linking agent, 6 mg (0.04 mmol) AIBN as the initiator, 4 mg (0.02 mmol) DNT as the target molecule and 0.6 mL ethanol as the solvent, to yield MIP-PMMAA. The polymerization was photoinitiated by UV light at wavelength of 365 nm, for 5 hours at room temperature. Silica particles were etched by 5% hydrofluoric acid bath and completely removed from the polymer. Finally, the DNT molecules were removed by ethanol.

Amino functionalized carbon dots (AC-dots) were synthesized following a published method [31]. Covalent bonding was formed between the porous MIP films and AC-dots in a 0.033 mM EDC/0.033 mM NHS aqueous solution.

4.3. Characterization

Fourier Transformed Infrared Spectroscopy (FTIR) spectrum of AC-dots was collected with a Nicolet 4700 FT-IR in order to identify the functional groups present on the surface of AC-dots.

Swelling experiments of NIP-PMMAA were performed in: (1) phosphate buffer at three different pH values (4, 7 and 10) at room temperature; and (2) NaCl or CaCl₂ solution at different ionic strengths (1, 10 and 100 mM) at room temperature. The polymers were swollen in solutions for 48 h at ambient temperature and the degree of swelling was determined gravimetrically. At least three samples of NIP-PMMAA material was tested under each condition.

4.4. Fluorescence quenching experiments

Hitachi F-4500 Fluorescence Spectrophotometer was used for photoluminescent properties. Emission scans were performed from 300 nm to 700 nm at a speed of 1200 nm * minute⁻¹ with the slit width of 5 nm at an excitation wavelength of 350 nm.

The initial FL intensity of MIP and NIP films was measured in DI. Secondly, sensor strips were dipped in the corresponding test solutions and incubated for 30 minutes, as determined in previous research [16]. Then, sensors were rinsed with DI water and their FL intensities were measured submerged in pure water at neutral pH. The level of quenching is calculated as the ratio of the initial intensity to the intensity measured after exposure to the sample, F₀/F. The linear relationship between F₀/F and DNT concentration in solution followed Stern–Volmer equation [28].

$$F_0/F = 1 + K_{sv}C_q, \quad (5)$$

where F₀ and F are the fluorescence intensity in the absence and presence of quencher, respectively, C_q is the concentration of the quencher, and K_{sv} is the quenching constant for the quencher. At least three MIP or NIP films were tested under each conditions; FL intensity measurement was conducted at least three times for each sample.

For all test solutions, DNT concentrations were determined by high-performance liquid chromatography (HPLC) coupled with a 15-cm by 4.6-mm Epic C18 column. Methane/water (50:50, v/v) was used as the mobile phase at flow rate of 0.75 mL·min⁻¹ with UV Detection at 254 nm and injection volume of 10 μL.

4.5. Investigations on the effects of interferences

The effect of pH on the detection and quantification of DNT by the MIP sensor was investigated in 10 ppm DNT solutions adjusted at three levels of pH (4, 6, 9), adding HCl (10 mM) or NaOH (10 mM) solutions as needed. The DNT concentration was chosen because it falls into the linear range of the sensor detection range and causes a significant FL decrease. For comparison, the responses of MIPs and AC-Dots (0.2 μM) to pH, both having the similar FL intensities at neutral pH, were tested in

absence of DNT as well. The IS effect was investigated in 10 ppm DNT test solutions with a background concentration of at 1, 10, and 100 mM NaCl or CaCl₂.

Organic materials are ubiquitous in natural waters, originating from the decay of plants, animals or anthropogenic pollution. Natural organic matter (NOM) includes substances that can emit or quench fluorescence, as well as adsorb onto the sensor surfaces, and therefore induce error in sensor output. In order to assess this effect, experiments were performed with NIP and MIP sensors in mixture solutions of 10 ppm DNT with different levels of Suwannee River NOM (SRNOM), as a model of natural organic matter, at concentration of 1, 10 and 50 ppm. Control tests performed with NIP and MIP sensors at the same SRNOM concentration levels in the absence of DNT.

Finally, the sensor was tested in real water samples collected from Stephens Lake, Columbia, Missouri, and tap water at the University of Missouri (coming from Water Treatment Plant with Columbia Utilities) that were spiked with DNT. Immediately after collection, the sampled water was characterized by pH, conductivity and total organic carbon (TOC) measurements using a Shimadzu TOC-VCPN analyzer.

The MIP-sensor was dipped in the spiked solutions, left to equilibrate for 30 minutes and rinsed with DI before reading fluorescence intensity. DNT concentrations were determined by the quenching amount as established by the Stern-Volmer equation. Sensor measurements were validated by analytical determinations of the DNT concentrations in the samples, by high-performance liquid chromatography (HPLC).

Data availability statement

Supporting data is available via the University of Missouri's digital institutional repository (MOspace) web site at <https://mospace.umsys.edu>.

Declarations

Author contribution statement

Jingjing Dai: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Maria F. de Cortalezzi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- [1] O.Y. Henry, D.C. Cullen, S.A. Piletsky, Optical interrogation of molecularly imprinted polymers and development of MIP sensors: a review, *Anal. Bioanal. Chem.* 382 (2005) 947–956.
- [2] S. Li, Y. Ge, S.A. Piletsky, J. Lunec, *Molecularly Imprinted Sensors: Overview and Applications*, Elsevier, 2012.
- [3] G. Pan, Q. Guo, C. Cao, H. Yang, B. Li, Thermo-responsive molecularly imprinted nanogels for specific recognition and controlled release of proteins, *Soft Matter* 9 (2013) 3840–3850.
- [4] Y. Kanekiyo, R. Naganawa, H. Tao, pH-Responsive molecularly imprinted polymers, *Angew. Chem. Int. Ed.* 42 (2003) 3014–3016.
- [5] H.G. Wu, et al., A novel ion-imprinted hydrogel for recognition of potassium ions with rapid response, *Polym. Adv. Technol.* 22 (2011) 1389–1394.
- [6] P. Kueseng, M.L. Noir, B. Mattiasson, P. Thavarungkul, P. Kanatharana, Molecularly imprinted polymer for analysis of trace atrazine herbicide in water, *J. Environ. Sci. Health, Part B Pesticides* 44 (2009) 772–780.
- [7] D. Davoodi, M. Hassanzadeh-Khayyat, M.A. Rezaei, S.A. Mohajeri, Preparation, evaluation and application of diazinon imprinted polymers as the sorbent in molecularly imprinted solid-phase extraction and liquid chromatography analysis in cucumber and aqueous samples, *Food Chem.* 158 (2014) 421–428.
- [8] T. Alizadeh, M. Zare, M.R. Ganjali, P. Norouzi, B. Taviana, A new molecularly imprinted polymer (MIP)-based electrochemical sensor for monitoring 2,4,6-trinitrotoluene (TNT) in natural waters and soil samples, *Biosens. Bioelectron.* 25 (2010) 1166–1172.
- [9] T. Hao, et al., An eco-friendly molecularly imprinted fluorescence composite material based on carbon dots for fluorescent detection of 4-nitrophenol, *Microchim. Acta* 183 (2016) 2197–2203.
- [10] T.P. Rao, K. Prasad, R. Kala, J.M. Gladis, Biomimetic sensors for toxic pesticides and inorganics based on optoelectronic/electrochemical transducers—an overview, *Crit. Rev. Anal. Chem.* 37 (2007) 191–210.
- [11] Y. Wen, et al., Molecularly imprinted matrix solid-phase dispersion coupled to micellar electrokinetic chromatography for simultaneous determination of triazines in soil, fruit, and vegetable samples, *Electrophoresis* 33 (2012) 2454–2463.
- [12] Y.M. Yin, et al., Dummy molecularly imprinted polymers on silica particles for selective solid-phase extraction of tetrabromobisphenol A from water samples, *J. Chromatogr. A* 1220 (2012) 7–13.
- [13] S. Han, S.T. Mukherji, A. Rice, J.B. Hughes, Determination of 2,4- and 2,6-dinitrotoluene biodegradation limits, *Chemosphere* 85 (2011) 848–853.
- [14] E.M. Lent, L.C.B. Crouse, M.J. Quinn Jr, S.M. Wallace, Assessment of the in vivo genotoxicity of isomers of dinitrotoluene using the alkaline comet and peripheral blood micronucleus assays, *Mutat. Res.* 742 (2012) 54–60.
- [15] Agency, U. S. E. P. Office of solid waste and emergency response, Technical Fact Sheet – Dinitrotoluene (DNT). Vol. EPA 505-F-14-010, 2014.
- [16] J. Dai, X. Dong, M.F. de Cortalezzi, Molecularly imprinted polymers labeled with amino-functionalized carbon dots for fluorescent determination of 2, 4-dinitrotoluene, *Microchimica Acta* 184 (2017) 1369–1377.
- [17] S. Wagner, J. Bell, M. Biyikal, K. Gawlitza, K. Rurack, Integrating fluorescent molecularly imprinted polymer (MIP) sensor particles with a modular microfluidic platform for nanomolar small-molecule detection directly in aqueous samples, *Biosens. Bioelectron.* 99 (2018) 244–250.
- [18] M. Bayati, J. Dai, A. Zambrana, C. Rees, M. Fidalgo de Cortalezzi, Effect of water chemistry on the aggregation and photoluminescence behavior of carbon dots, *J. Environ. Sci.* 65 (2018) 223–235.
- [19] Y. Dong, et al., Polyamine-functionalized carbon quantum dots as fluorescent probes for selective and sensitive detection of copper ions, *Anal. Chem.* 84 (2012) 6220–6224.
- [20] O. Okay, *General Properties of Hydrogels*, vol. 6, Springer, 2009.
- [21] W. Chen, et al., Molecularly imprinted polymers with stimuli-responsive affinity: progress and perspectives, *Polymers* 7 (2015) 1689–1715.
- [22] C. Ding, A. Zhu, Y. Tian, Functional surface engineering of C-dots for fluorescent biosensing and in vivo bioimaging, *Accounts Chem. Res.* 47 (2014) 20–30.
- [23] H. Kempe, M. Kempe, Influence of salt ions on binding to molecularly imprinted polymers, *Anal. Bioanal. Chem.* 396 (2010) 1599–1606.
- [24] J. Huang, X. Hu, W. Zhang, Y. Zhang, G. Li, pH and ionic strength responsive photonic polymers fabricated by using colloidal crystal templating, *Colloid Polym. Sci.* 286 (2008) 113–118.
- [25] J.R. Lakowicz, B.R. Masters, *Principles of fluorescence spectroscopy*, J. Biomed. Opt. 13 (2008), 029901.
- [26] J.G. Hering, *Principles and Applications of Aquatic Chemistry*, John Wiley & Sons, 1993.
- [27] E.P. Agency, Method 8330b, Nitroaromatics, Nitramines, and Nitrate Esters by High Performance Liquid Chromatography (HPLC), 2006.
- [28] M.E.K. Wahba, N. El-Enany, F. Belal, Application of the Stern–Volmer equation for studying the spectrofluorimetric quenching reaction of eosin with clindamycin hydrochloride in its pure form and pharmaceutical preparations, *Anal. Method* 7 (2015) 10445–10451.
- [29] W. Stöber, A. Fink, E. Bohn, Controlled growth of monodisperse silica spheres in the micron size range, *J. Colloid Interface Sci.* 26 (1968) 62–69.
- [30] P. Jiang, J. Bertone, K. Hwang, V. Colvin, Single-crystal colloidal multilayers of controlled thickness, *Chem. Mater.* 11 (1999) 2132–2140.
- [31] M. Zheng, et al., Integrating oxaliplatin with highly luminescent carbon dots: an unprecedented theranostic agent for personalized medicine, *Adv. Mater.* 26 (2014) 3554–3560.