Supramolecular Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713649759

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To cite this Article Maynor, Marc S. , Deason, Travis K. , Nelson, Toby L. and Lavigne, John J.(2009) 'Multidimensional response analysis towards the detection and identification of soft divalent metal ions', Supramolecular Chemistry, 21: 3, 310 — 315

To link to this Article: DOI: 10.1080/10610270802516658

URL: http://dx.doi.org/10.1080/10610270802516658

Supramolecular Chemistry
Vol. 21, Nos. 3–4, April–June 2009, 310–315

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Multidimensional response analysis towards the detection and identification of soft divalent metal ions

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(*Received 27 July 2008; final version received 17 September 2008*)

The stepwise detection and identificational of soft divalent metal ions is reported using a carboxylate functionalised poly(thiophene) in combination with small-molecule competitive binders and multivariate statistics. This approach involves two sensory events. First, the addition of polyvalent metal ions (alkaline-earth metals, transition metals and lanthanides) leads to precipitation of the polymer in a qualitative detection step. Then, the addition of a competitive ligand (α,ω-diamines) causes the precipitate to re-dissolve for soft divalent metal ions. Unique colorimetric fingerprints are obtained for different analytes, allowing for facile identification based on statistical analysis of the spectral response from the ensemble, i.e. multidimensional response analysis. In a highly competitive aqueous media, identification of divalent metal ions was achieved with 100% accuracy. In a parallel investigation, mercury salts did not cause precipitation but did alter the fluorescence response of polymer **1** producing a Stern–Volmer constant (K_{sv}) of $1.0 \times 10^5\,M^{-1}$.

Keywords: poly(thiophene); pattern recognition; sensor; metal detection; fluorescence

1. Introduction

Previously, we have demonstrated that the multidimensional response from a single, cross-reactive conjugated polymer (*1*) or series of cross-reactive conjugated polymer–small-molecule hybrids (*2*) can be used to identify and quantify structurally similar and biologically relevant analytes. This analysis has used statistical analysis to probe the full spectral response from the systems to gain more information than is afforded from a simple single wavelength measurement or even from ratiometric analyses. Herein, we advance this methodology for the detection and identification of soft divalent metal ions.

Facile, sensitive and reliable methods for detecting metal ions are important for quality control (*3*) and environmental science and technology (*4*). Therefore, considerable efforts have been devoted to the design and synthesis of chemosensors that are capable of detecting metal ions (*5*). Macromolecular targets such as crown ethers (*6*) and conjugated polymers (*7*) have been used for trace metal detection. Conjugated polymers are particularly attractive for sensing applications, because they contain built-in transduction mechanisms (producing colorimetric, fluorescent or electronic variations) that are highly sensitive to external stimuli that cause structural perturbations and electron density changes within the polymer backbone (*8*). Metal ions typically influence a conjugated polymer by ion-induced conductivity fluctuations which lower the charge carrier mobility (electrostatic effects) or by destroying or enhancing the effective conjugation length of the polymer (conformational change) (*9*).

When discriminating metal ions using conjugated polymers, most look for large optical differences (*10*), while few have paid attention to more subtle spectral changes (*11*). The combination of supramolecular chemistry with pattern recognition, aptly called supramolecular analytical chemistry (*12*), provides an avenue in which enhanced classification of target analytes can be collected from large and small optical responses of an array of chemosensors (*13*). We have advanced this concept and used the previous colorimetric multidimensional response from a cross-responsive carboxylate functionalised poly(thiophene) coupled with pattern recognition protocols to identify and differentiate structurally similar (*1a*) and biologically relevant amines (*1b,c*). This approach relied on numerous dynamic polymer–analyte interactions causing main-chain conformational changes, π–π stacking between polymer chains and scattering of visible light caused by the solution stable polymer–analyte aggregates. Herein, we take advantage of the multidimensional response from a polymer–small-molecule ensemble to extend this paradigm to detect and identify a series of divalent metal ions. In this analysis, the divalent metals cause precipitation of the polyvalent poly(thiophene) indicating the presence of a target analyte (i.e. *detection*). Upon addition of a small molecule diamine

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ISSN 1061-0278 print/ISSN 1029-0478 online

DOI: 10.1080/10610270802516658
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that can effectively compete for metal binding, the polymer–metal aggregate is resolubilised and the analyte is *identified* based on a statistical analysis of the colorimetric response from the polymer–metal–diamine composite.

2. Experimental

2.1 General remarks

Polymer **1** was synthesised following literature procedures (*1*). The amines used in the assays were obtained from Sigma-Aldrich and used without further purification. The metal chlorides used in these studies were purchased from Acros and used as received. Plastic (polypropylene) microtitre plates were purchased from Fisher (Pittsburgh, USA).

2.2 Instrumentation

Absorbance studies were performed using a SPECTRAmax Plus plate reader from Molecular Devices. Fluorescence studies were carried out on a SPECTRAmax GEMINI EM plate reader from Molecular Devices. All measurements were carried out with a Peltier thermostated sample holder maintained at 25°C.

2.3 Assay conditions

Stock solution preparation. Deionised water was used for all aqueous studies. The buffer (50 mM HEPES buffer solution) was prepared by dissolving 23.83 g of HEPES into 1 L of deionised water. The pH was then adjusted to 7.4 by using 1 M NaOH and 1 M HCl using a Thermo Orion pH metre and filled to the final volume of 2 l. Preparation of 10 ml of 100 mM diamine stock solutions was achieved by dissolving 67.5 μl (0.96 mmol) of 1,2-diaminoethane (EDA) or 84.0 μl (1.24 mmol) of 1,3-diaminopropane (PDA) in 10 ml of 50 mM HEPES buffer solution (pH 7.4). The polymer solution was prepared by adding 61.6 mg of **1** to 80 ml of deionised water and the pH was adjusted to 7.4 using 1 M NaOH and 1 M HCl and then sonicated for 30 min. This preparation produces 80 ml of a 5 mM reddish/purple working polymer solution. A 20 mM stock solution of each metal ion used in his study was prepared by dissolving 47.5 mg (0.19 mmol) of $CoCl_2 \cdot 6H_2O$, 47.5 mg (0.19 mmol) of $NiCl_2 \cdot 6H_2O$, 34 mg (0.20 mmol) of $CuCl_2 \cdot 2H_2O$, 36.6 mg (0.20 mmol)of $CdCl_2$, 55.6 mg (0.20 mmol) of $PbCl_2$, 40.6 mg (0.15 mmol) of $MnCl_2 \cdot 6H_2O$, or 27.2 mg (0.19 mmol) of $ZnCl_2$ into 10 ml of deionised water.

2.4 Analytical methods

2.4.1 Conditions for the UV/vis studies of polymer **1** response to metal ions and diamines

The 5 mM stock solution of polymer **1** (pH 7.4) was loaded at 24 μl per well into a multi-well plate. Then, 3 μl of a 20 mM solution of the desired metal ion was added followed by 3 μl of a 100 mM solution of either EDA or PDA. Finally, the wells were filled to the total volume of 300 μl with 50 mM HEPES buffer solution (final concentration 40 mM). The final concentrations of polymer **1**, the metal ions and the diamines were 400, 200 and 1000 μM, respectively. The absorbance was measured from 350 to 700 nm for each of 24 replicates.

2.4.2 Conditions for fluorescence studies of polymer **1** response to $HgCl_2$

A 5 mM stock solution of polymer **1** (pH 7.4) was loaded at 24 μl per well into a multi-well plate. To each well was added aliquots of the stock $HgCl_2$ solution to afford final concentrations of 0.2 mM (6 μl of 10 mM $HgCl_2$), 0.5 mM (15 μl of 10 mM $HgCl_2$), 1 mM (30 μl of 10 mM $HgCl_2$), 5 mM (15 μl of 100 mM $HgCl_2$) and 10 mM (30 μl of 100 mM $HgCl_2$). A total volume of 300 μl was reached for each well upon addition of 50 mM HEPES buffer solution (final concentration 40 mM). The fluorescence was measured from 500 to 800 nm ($\lambda_{ex} = 450$ nm) for each of 16 replicates.

2.5 Data analysis

Absorbance data were analysed using the commercially available feature selection algorithm, linear discriminant analysis (LDA), from Systat (*14*). This software was used to obtain the general and leave-one-out cross-validation classification accuracies; whereas, Statistica was used as a graphical program. For the LDA, each spectrum was preprocessed by dividing each spectral intensity by the sum of all the spectral intensity in that spectrum. This normalisation made the area under the curve equal to one. The assay was analysed using wavelengths between 350 and 700 nm chosen every 10 nm from the spectral response for each polymer–metal–amine solution at each concentration. The data for polymer **1** and the metal ions response in the presence of EDA were placed into the Systat worksheet and then the data from polymer **1** and the metal ions response with PDA were included in the series, such that the two spectra were linked end-to-end to provide 72 inputs (36 wavelengths from each diamine-based spectrum). Classification accuracies were based on leave-one-out cross-validation and thus, the estimates of the classification for each sample were independent of the discriminant model.

3. Discussion

Figure 1 displays our general approach towards detecting and identifying divalent metal ions. To simply illustrate the applicability of this approach, three divalent metal

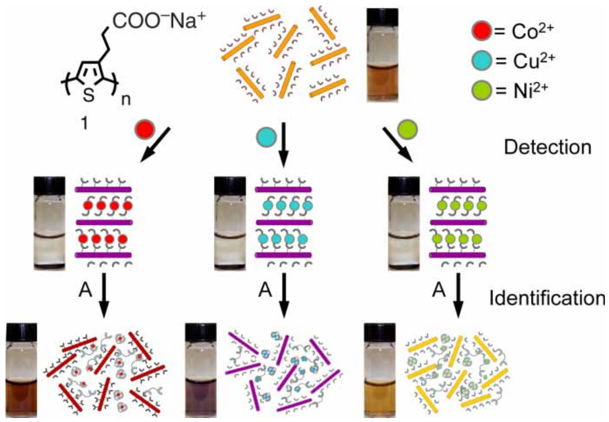

Figure 1. Schematic depicting how detection and identification of metal ion occurs, including images of the solutions after each addition. Addition of metal ions (coloured balls) to polymer **1** (yellow rods) aggregates the polymer (purple rods) resulting in detection of the ions. Addition of competitive binding diamines (A) produces soluble supramolecular assemblies with different colorimetric responses.

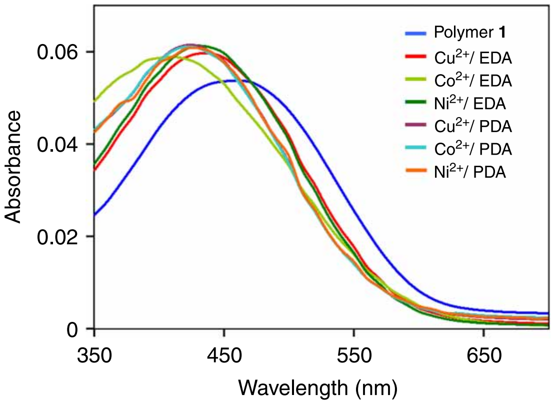

Figure 2. Normalised absorbance spectra of polymer **1** (400 μM) and the response of the polymer **1**/M^{2+} ($M^{2+} = 200$ μM; M = Co, Cu and Ni) in the presence of 1,2-diaminoethane (EDA, dark grey) and 1,3-diaminopropane (PDA, light grey) (1000 μM) in 40 mM HEPES buffer (pH 7.4).

chlorides, $CoCl_2$, $CuCl_2$ and $NiCl_2$, were used in the initial studies. This approach is advantageous because there are two sensory events that allow for effective detection of divalent metal ions. The first sensory event involves the addition of aqueous solutions of divalent metal ions to a buffered solution (40 mM HEPES, pH 7.4) of HT-2,5-poly(thiophene-3-propionate) (**1**). It has been postulated that the metal ion aligns the polymer into ordered rods (*15*) leading to rapid precipitation of a brown/purple polymer–metal aggregate, caused by ionic cross-linking of the polymer chains with the divalent metal. The second sensory event occurs upon addition of a ligand that can compete for binding of the metal with the carboxylate of the poly(thiophene), such as EDA or PDA. This competitive binding causes the polymer–metal aggregate to disassociate and return to the solution. From this, several dynamic colour producing interactions are produced including: (1) coordinated metal–diamine complexes; (2) size-dependent counterion-induced main-chain conformational changes of the polymer, where the counterion may be the metal ion, the ammonium analyte or some amine–metal adduct and (3) modified inter-chain interactions between polymers resulting from bridging interactions with the ditopic diamines or the divalent metal ion. The diverse variations in colour allow for identification of the metal ions.

At relatively high metal ion concentrations (~1 mM), discrimination between Co^{2+}, Cu^{2+} and Ni^{2+} is obvious to the naked eye (vials at the bottom of Figure 1). To further demonstrate the utility of this system, however, we decided to investigate lower metal ion concentrations in a highly competitive, buffered aqueous environment. Figure 2 shows the normalised absorbance spectral response of polymer **1** (400 μM) after the addition of either $CoCl_2$, $CuCl_2$ or $NiCl_2$ (200 μM each) followed the addition of either EDA or PDA in 40 mM HEPES buffer solution (pH 7.4). At lower metal ion concentrations, there are reduced metal–diamine interactions leading to less variation in colour, making naked-eye discrimination difficult. However, there are subtle, yet clear spectral differences depending on the metal and amine used. As seen in Figure 2, the differences between spectral traces are even difficult to distinguish between, still using pattern recognition protocols these subtle variations can be delineated.

Linear discriminant analysis was employed to reduce noise and simplify the spectral fingerprint response. To ensure reproducibility, 24 replicates of the response from the polymer-diamine (EDA or PDA) ensemble to each metal ion (72 total replicates) were collected on separate days using separate stock solutions. The pH was controlled to ensure that discrimination was based upon the response to the metal ion rather than simple pH fluctuations. The absorption spectra were recorded using a microtitre plate reader with samples randomised on the plates to avoid systematic errors. Analysis was performed on the response of the polymer–metal–diamine ensemble across the entire spectrum between 350 and 700 nm every 10 nm (36 total wavelengths).

Figure 3 shows the two-dimensional projection of the LDA results for polymer **1** in conjunction with EDA and PDA, in parallel, responding to 200 μM of cobalt, copper and nickel salts. Each point in the plot is an individual replicate that contains information from the 72 wavelengths from the representative spectra (36 wavelengths from each spectra associated with EDA and PDA). Therefore, each axis of the LDA plot represents the weighted combinations of the 72-dimensional data.

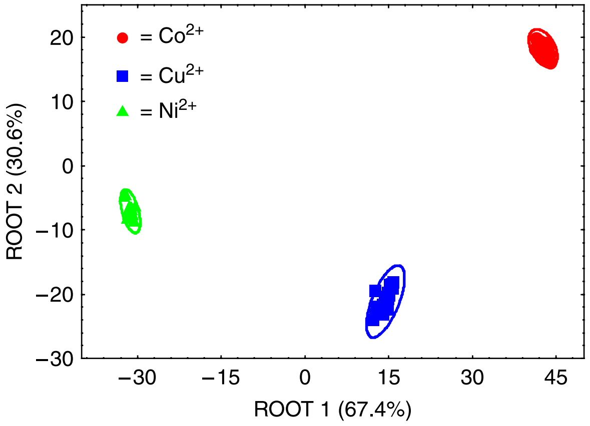

Figure 3. Two-dimensional LDA plot of the response from 400 μM polymer **1** responding to 200 μM $CoCl_2$, $CuCl_2$ and $NiCl_2$ in the presence of 1000 μM of 1,2-diaminoethane or 1,3-diaminopropane in parallel measurements and serial analysis. Ellipses indicate 99% confidence interval for 24 replicates each.

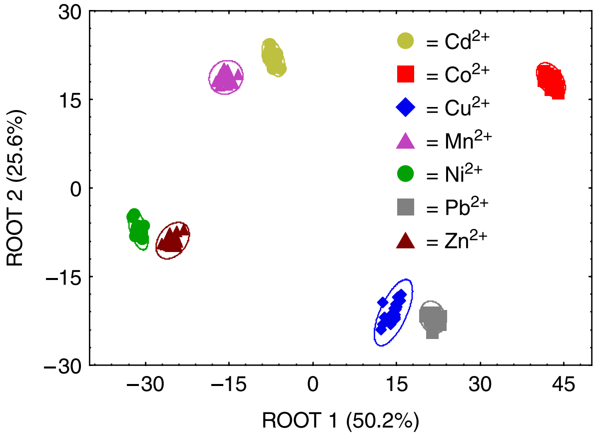

Figure 4. Two-dimensional LDA plot of the response of polymer **1** (400 μM) towards divalent metals (200 μM) in the presence of 1,2-diaminoethane or 1,3-diaminopropane (1000 μM) in parallel measurements and serial analysis, in 40 mM HEPES buffer. Ellipses indicate 99% confidence interval for 24 replicates each.

Leave-one-out cross-validation was used to estimate the predictive ability of the LDA model and showed excellent discrimination between the metal ions accurately identifying the analyte 100% of the time (72/72 samples). It is noteworthy to mention that this discrimination is achieved in highly competitive aqueous media; specifically, 50 μM metal ions are identified in 40 mM HEPES buffer.

Given the impressive results for the above initial studies, we were encouraged to push the limits of this analysis and therefore carried out further studies to evaluate the breadth of this approach. To this end, a series of divalent and trivalent metal chlorides including alkaline-earth metals such as $MgCl_2$, $CaCl_2$ and $BeCl_2$, lanthanides including $LaCl_3$, $EuCl_3$ and $TbCl_3$, and late-transition metals such as $CdCl_2$, $ZnCl_2$ as well as $PbCl_2$ were examined using the conditions described above. While all of these metal chlorides led to the rapid precipitation of polymer **1**, only the polymer precipitates resulting from certain late-transition metals were able to be brought back into solution upon addition of EDA or PDA. We reasoned that alkaline-earth metals and the lanthanides are 'hard metals' and binds more strongly to the carboxylate of the polythiophene, whereas the late-transition metals are 'soft' and chelate preferentially to the diamines rather than the carboxylate of the poly(thiophene).

To assess our ability to differentiate between these softer transition metals, 24 replicates of each of the seven divalent metal chlorides ($CoCl_2$, $CuCl_2$, $NiCl_2$, $ZnCl_2$, $CdCl_2$, $MnCl_2$ and $PbCl_2$) were examined as described above. Figure 4 shows the two-dimensional LDA plot of the polymer **1** response to the studied metal ions in the presence of EDA or PDA. Leave-one-out cross-validation analysis again showed outstanding discrimination between the metal ions and correctly identified each analyte 100% of the time (168/168 total samples).

During these studies, it was found that precipitation of polymer **1** did not occur upon addition of $HgCl_2$ even at 25 equivalent excess. Given this unique response, further studies were carried out to probe the utility of polymer **1** towards sensing mercury. Since the optical properties of conjugated polymers are sensitive to minor changes in conformation as well as possible intra- and inter-chain energy transfer, we reasoned that mercury could modify the fluorescence from polymer **1**, thereby signalling binding. For the precipitation of polymer **1** with other polyvalent metal ions as well as other conjugated polyelectrolyte-based sensors (*16*), the key feature is the binding of the negatively charged carboxylate on the polymer to the oppositely charged cation. However, given the lack of precipitation of polymer **1** upon addition of mercury salts and coupled with the thiophilic nature of mercury (*17*), we reasoned that a change in binding might exist here and it is likely that the mercury actually binds with the sulphur of the thiophene ring and very weakly to the carboxylate of the poly(thiophene).

Figure 5(a) illustrates the effect of the addition of increasing amounts of $HgCl_2$ to polymer **1**. Hg^{2+} was found to be an efficient quencher of polymer **1**. A Stern–Volmer analysis was used to measure the quenching efficiency of mercury on polymer **1**. (The Stern–Volmer constant (K_{sv}) is defined by: $F_0/F = K_{sv}[Q] + 1$, where F_0 is the intensity of the fluorescence without the addition of any quencher (defined as Q). F is the intensity of the fluorescence in the presence of a given concentration of Q (*18*).)

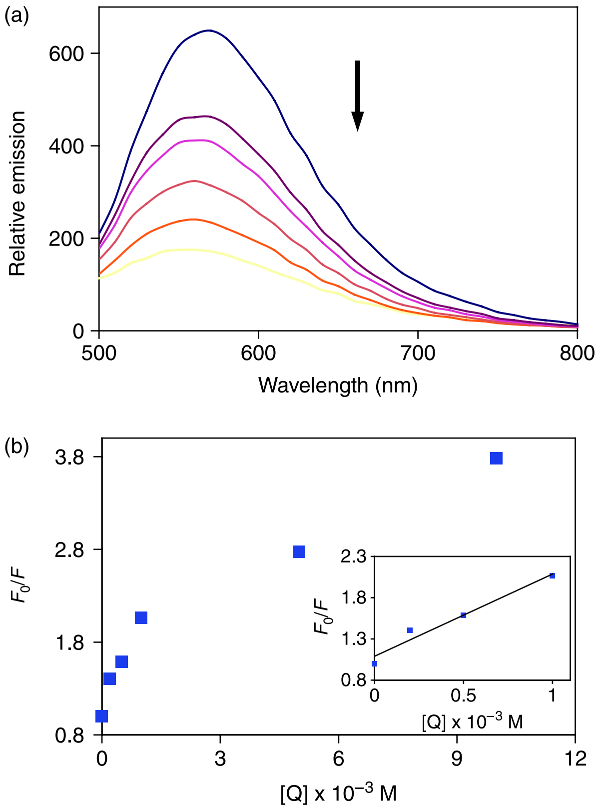

Figure 5. (a) Emission spectra ($\lambda_{ex} = 450$ nm) of a 0.4 mM solution of polymer **1** in 40 mM HEPES buffer at pH 7.4 in response to increasing concentrations of mercury ions. (b) Plot of F_0/F of polymer **1** at 570 nm versus mercury ion concentration. The inset shows the best fit for the linear portion of the F_0/F plot ($y = 0.9972x + 1.0897$; $R^2 = 0.9634$), producing a Stern–Volmer constant (K_{sv}) for Hg^{2+} ions of $1.0 \times 10^5\,M^{-1}$.

Though this analysis does not differentiate between static quenching or simple heavy atom quenching mechanisms, the Stern–Volmer constant, K_{sv}, is a good indicator of the interaction between Hg^{2+} and polymer **1** (*19*). The larger the K_{sv} value indicates a greater sensitivity of the system towards the quencher. The F_0/F curve in Figure 5(b) displays saturation dynamics. The inset in Figure 5(b) shows the linear region of the F_0/F plot for polymer **1** versus the concentration of Hg^{2+} ions. The apparent K_{sv} value was found to be $1.0 \times 10^5\,M^{-1}$ when polymer **1** was exposed to mercuric ions. This value is comparable to the one reported by Bunz and co-workers, where they used a carboxylated poly(*p*-phenyleneethynylene) in tandem with proteins to examine the sensory response of the ensemble towards mercury ions (*20*).

Figure 6 depicts the proposed mechanism for the quenching of polymer **1** in the presence of Hg^{2+} ions. We propose that a self-assembled loose network of fluorescent poly(thiophene) chains are dynamically assembled upon addition of Hg^{2+} ions. The mercury ions interact with the sulphur of the thiophene rings and carboxylates inter- and intra-molecularly, which may cause π–π stacking of the polymer main chains along with twisting of the conjugated polymer backbone. Importantly, these interactions must be weak and dynamic because precipitation does not occur. Similar interactions have shown to have a profound effect on the fluorescence of a carboxylated substituted poly(*p*-phenyleneethynylene) (*20*).

In summary, in this proof of principle study, a carboxylate functionalised conjugated polymer has been used for the stepwise detection and identification of soft metal ions. In the first step, precipitation of polymer **1** upon addition of divalent metal ions led to a quick semi-qualitative detection. Addition of competing ligands such as EDA or PDA led to the immediate dissolution of the polymer–metal aggregates. The precipitates resulting from the addition of alkaline-earth metals, early transition metals and the lanthanides did not re-dissolve upon addition of diamine providing an opportunity to readily differentiate these classes of metal ions from softer analytes. For those aggregates that re-dissolved, the optical responses of polymer **1** upon addition of the respective metal ion with EDA and PDA was statistically analysed using LDA to identify the analyte.

Interestingly, mercury did not cause precipitation but did alter the optical properties of polymer **1** suggesting some interaction between the polymer and metal ion. Concentration dependence studies showed that mercury is an efficient quencher of polymer **1** with a Stern–Volmer constant (K_{sv}) of $1.0 \times 10^5\,M^{-1}$. We are currently investigating these approaches for the detection and discrimination of mixtures of metal ions as well as detection of mercury ions in fish.

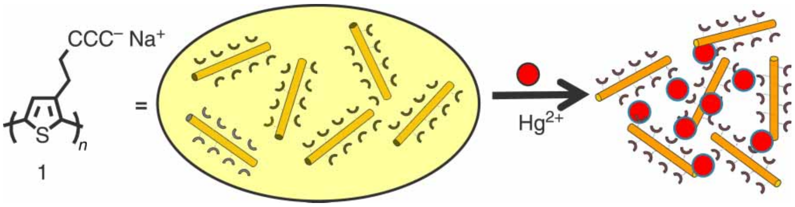

Figure 6. Proposed mechanism of the quenching effects shown by polymer **1** upon addition of mercuric ions.

Acknowledgements

We gratefully acknowledge the support for this work from the Research Corporation. M.S.M. and T.L.N. acknowledge SREB for dissertation year fellowships. T.K.D. acknowledge the University of South Carolina for the Undergraduate Magellan Fellowship. M.S.M. thanks the University of South Carolina Graduate School for the Summer Dissertation Fellowship.

Supporting Information, available online. Detailed statistical outputs used in the assay.

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