

# Dendrimer Precursors for Nanomolar and Picomolar Real-Time Surface Plasmon Resonance/Potentiometric Chemical Nerve Agent Sensing Using Electrochemically Crosslinked Ultrathin Films\*\*

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Nanomolar detection and specific recognition of pinacolyl methylphosphonate (PMP), a hydrolysis product and an analog of a relatively persistent class of toxic nerve agents, has been achieved. In addition, picomolar sensitivity is observed with methylphosphonic acid (MPA), an end-hydrolysis product for all organophosphate-based nerve agents. This is achieved using a combined surface plasmon resonance (SPR) and potentiometry setup. A modified polyamidoamine (PAMAM) carbazole/Cu<sup>2+</sup> dendrimer, which is electrochemically crosslinked on a self-assembled monolayer (SAM) modified Au substrate, is used as an active sensing element for trapping the nerve-agent analogs. The ultrathin films have been used to study the anchoring of nerve agents via non-covalent interactions. The carbazole to amine ratio is optimized to ensure free primary amines are available to interact with the analyte and the Cu<sup>2+</sup> ions present in the system, which further enhances the selectivity. The carbazole group on the periphery serves a dual purpose: crosslinking the dendrimers to form a conjugated network film, and generating the potentiometric response. The adsorption kinetics are monitored by using an in situ SPR/potentiometric setup. This technique not only offers a real-time dual detection of highly toxic nerve-agent analogs, but also shows viability for future sensor-device applications.

## 1. Introduction

Over the past two decades, the research and development of chemical sensors has been of prime interest for applications ranging from separations to remote sensing.<sup>[1]</sup> For chemical warfare and terrorism defense, there is a continuous need for new and improved methods for the detection of highly toxic organophosphate nerve agents.<sup>[2]</sup> The nerve agent, either as a gas, an aerosol, or a liquid, can penetrate the body through the skin. It can rapidly hydrolyze in water and soil to produce pinacolyl methylphosphonate (PMP), methylphosphonic acid (MPA), etc., as secondary analytes (Fig. 1).<sup>[3]</sup> Growing concerns over possible contamination of water supplies by nerve agents has also prompted the desire for small portable devices with rapid real-time sensing, capable of detecting trace amounts in water.<sup>[4]</sup> Current tools to determine these toxic materials rely on various gas- and liquid-chromatography techniques, which although very sensitive and quantitative, have disadvantages<sup>[5]</sup>—they are time consuming, non-portable, and not suit-

able for rapid analysis under field conditions.<sup>[6]</sup> To explore new methods, the sensing of nerve agents was performed using a combined surface plasmon resonance (SPR) spectroscopy technique<sup>[7]</sup> (Kretschmann configuration) and a potentiometric sensor assembly, as shown in Figure 2. The advantage of this approach is that both electrochemical and optical response can be monitored in situ, simultaneously, and rapidly. This dual sensing method is performed in real time, and can also be made portable through the modification of commercially available portable SPR instruments.

An amine-terminated generation four (G4) polyamidoamine (PAMAM) dendrimer was used in this study because this dendrimer is not only known to be rich in amide functionality, but also contains 64 peripheral primary amines. In principle, this peripheral functionality not only provides for peripheral modification, but can contribute towards forming hydrogen bonds and loading of Cu<sup>2+</sup> ions.<sup>[8]</sup> The PAMAM dendrimer itself is water soluble and hydrophilic, which is advantageous as a sensing element for detecting aqueous analyte species. However, it is necessary to affix the dendrimer to a surface capable of transduction, e.g., optical, electrical, or electrochemical, in a thin-film morphology. We modified the G4-PAMAM dendrimer using an electroactive carbazole group at the periphery (Scheme 1). The modification with carbazole enables one to generate thin films of the modified dendrimer via electrochemical polymerization. The polymerization in this case results in the formation of a highly crosslinked, conjugated polycarbazole film from a “dendrimer precursor”.<sup>[9]</sup> This thin film is very robust and stable in nature because it is infusible and insoluble in common organic solvents, making it suitable for sensor applications. One advantage of using the electroactive carbazole

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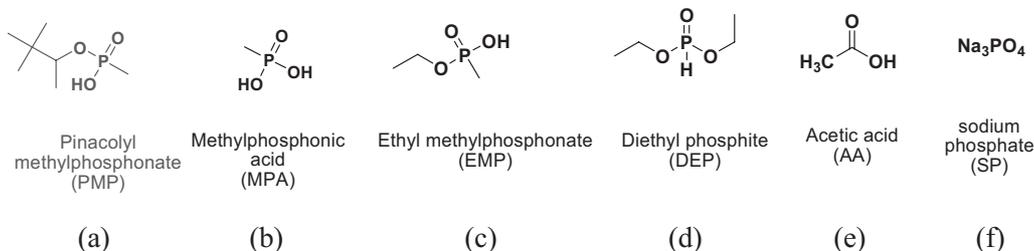


Figure 1. Various analytes used in the sensor study.

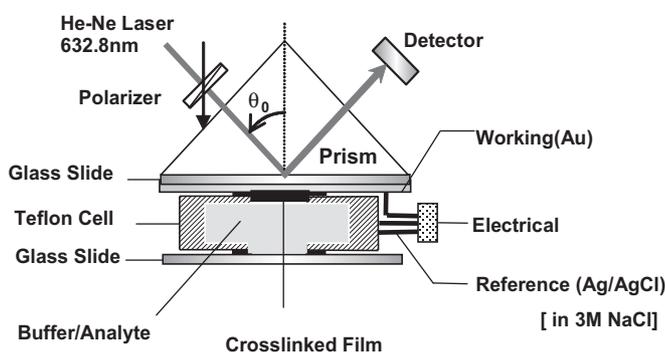
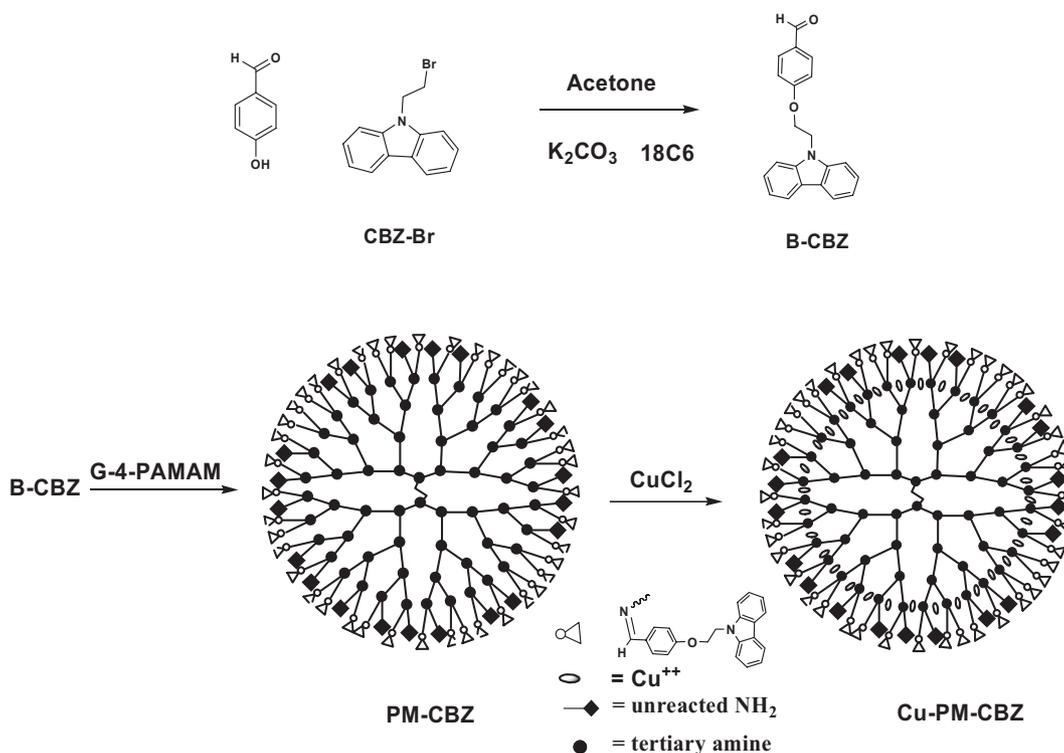


Figure 2. Sensor setup for in situ SPR/potentiometric measurements.

is that it can be crosslinked in the desired redox window without reducing the  $\text{Cu}^{2+}$  ions and therefore the activity of the metal ion is maintained in the film.

Conducting polymers have been used for chemical and biological sensing in a number of applications and device configurations.<sup>[10]</sup> However, very few studies have previously been reported using conducting polymers as nerve-agent sensors,<sup>[11]</sup> in particular polycarbazole, which has been shown to be useful for other sensor applications.<sup>[12a-c]</sup> In the present study, the formation of a polycarbazole conjugated network is advantageous in terms of its electrical property, which can be used to generate an efficient potentiometric response.<sup>[12d,e]</sup> The potentiometric transduction mode is a simple form of sensing, in which the conducting polymer serves as a signal transducer and



Scheme 1. Synthetic scheme for the carbazole grafting and  $\text{Cu}^{2+}$  complexation with PAMAM. CBZ: carbazole.

changes in the open-circuit potential (i.e., an electrochemical cell potential at zero current) are measured as the sensor signal. The open-circuit potential of a conductive polymer electrode depends on the redox state of the polymer. The redox state can be influenced by either injecting electric charge or adding a reagent or analyte of choice that interferes with the redox equilibrium.<sup>[13]</sup>

## 2. Results and Discussion

A simple synthetic approach was taken to modify G4-PAMAM dendrimers with carbazole (PM-CBZ), as shown in Scheme 1. The carbazole group should provide the electropolymerizability sufficient for the formation of a conjugated polymer network film.<sup>[10]</sup> The ratio of carbazole to amine present in the precursor dendrimer periphery was optimized in order to ensure enough free primary amines were left to interact with the analyte. At the same time, by loading Cu<sup>2+</sup> ions at the periphery of the dendrimer, it should form selective non-covalent interactions with the P=O bond (P=O...Cu<sup>2+</sup>) present in the nerve agent.<sup>[14]</sup> The presence of copper ions therefore not only makes this system selective, but can also assist in directly hydrolyzing the P-F bonds present in actual toxic nerve-gas agents.<sup>[15]</sup>

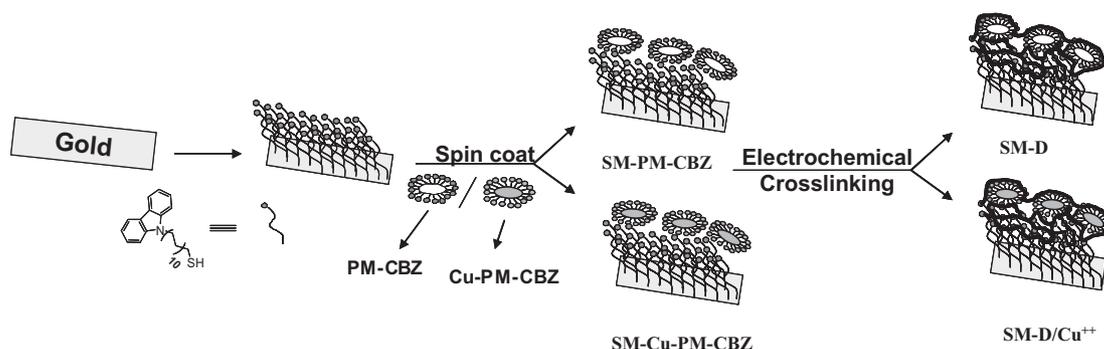
The loading of the Cu<sup>2+</sup> ions into the dendrimer was monitored using UV-vis spectrophotometric titration in solution as described in the Supporting Information (SI Fig. 1). An average of 32 bound Cu<sup>2+</sup> ions was determined on the copper-ion-modified dendrimer (Cu-PM-CBZ).<sup>[16a]</sup> The Cu<sup>2+</sup> binds to the two interior tertiary amines, with the other two positions being taken by weakly bound water or amide oxygens.<sup>[16b,c]</sup> This was the case as observed from the UV-vis spectra, where a peak at 900 nm due to the CuCl<sub>2</sub> is shifted to 703 nm by complexation with the dendrimer. The peak around 703 nm was found to be completely bleached and another peak at 735 nm was seen with the addition of PMP to the solution. After confirming the binding activity of the dendrimer/Cu<sup>2+</sup> complex in solution, further studies were carried out in terms of the film fabrication for this complex. The solution UV-vis studies are available in the Supporting Information.

Similar to our previous investigation on the formation of polymer films using the precursor polymer approach,<sup>[10]</sup> ultrathin films were prepared by spin-coating the solutions on bare Au and self-assembled monolayer (SAM) modified Au substrates. Both the PM-CBZ and Cu-PM-CBZ precursors were added at the same concentration (15.7 μM) to chloroform/toluene/methanol (9:1:2 v/v) and then spin-coated on 2.5 cm × 2 cm bare Au and SAM-modified (SM) Au substrates (Scheme 2). The electrochemical crosslinking was then performed in a three-electrode cell, with 0.1 M tetrabutylammonium hexafluorophosphate (TBAHFP) in 15 mL acetonitrile as supporting electrolyte, using the cyclic voltammetry (CV) technique. All potentials were measured versus Ag/Ag<sup>+</sup> (Supporting Information, SI Fig. 2). The redox window of 0.2–0.8 V was selected in order to prevent the reduction of Cu<sup>2+</sup> or oxidation of the SAM.

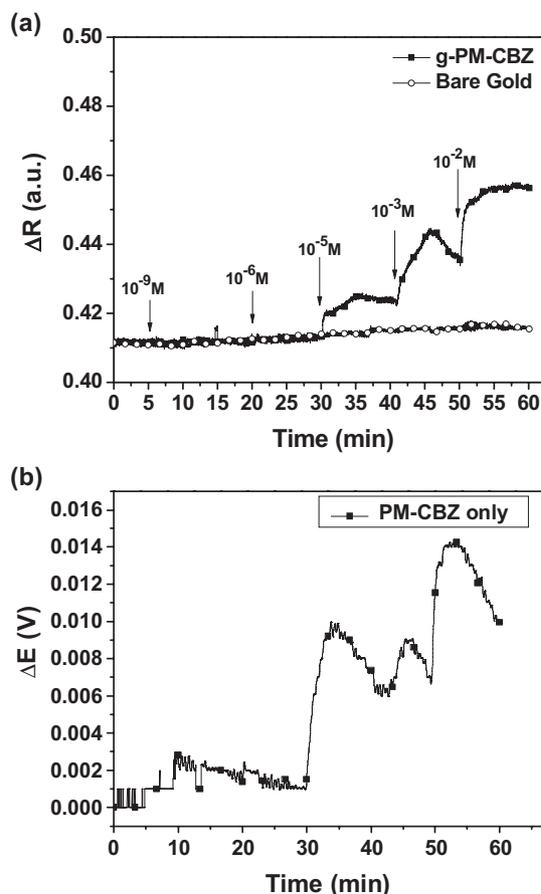
SPR angular-reflectivity scans of the spin-coated thin films revealed the presence of a smooth homogeneous film, as verified by atomic force microscopy (AFM) (Supporting Information SI Figs. 3 and 4). The total average thickness of the films, which includes the SAM as well as the dendrimer layer, were determined by fitting the experimental angular curves using an algorithm of the Fresnel equation.<sup>[7]</sup> The results showed a total average thickness of 12.4 and 15.2 nm for the SM-PM-CBZ and SM-Cu-PM-CBZ films, respectively, with the SAM accounting for 0.9 nm (see Supporting Information).

The sensor studies of the films were then performed using 20 mM ammonium acetate buffer (pH 7.2) and 0.1 M NaCl solution. It is known that the half-life of the PMP dramatically increases from 7 to 8 days at pH values close to (or below) 7.<sup>[17]</sup> Therefore the measurements were all performed while maintaining a constant pH 7.2. The change in potential (ΔE), where (ΔE) = observed potential (E<sub>o</sub>) – initial potential (E<sub>i</sub>), was recorded simultaneously as a function of time.

The initial SPR/potentiometric sensitivity studies were performed using spin-coated and electrochemically crosslinked PM-CBZ film on Au (g-PM-CBZ) and the bare Au film against varying concentrations of PMP (Fig. 3). This is in contrast to the SAM-modified Au substrate, which will be described later. The sensing experiment was performed by injecting different concentrations of PMP at regular intervals, as indicated by arrows in Figure 3a. As a reference, the bare Au showed no sig-



**Scheme 2.** Synthesis of the carbazole-terminated SAM and electrochemical crosslinking of the SAM and modified PAMAM–carbazole with and without copper ions.



**Figure 3.** a) SPR sensorogram for g-PM-CBZ and bare gold at different concentrations of the analyte PMP. b) The simultaneous potentiometric changes.

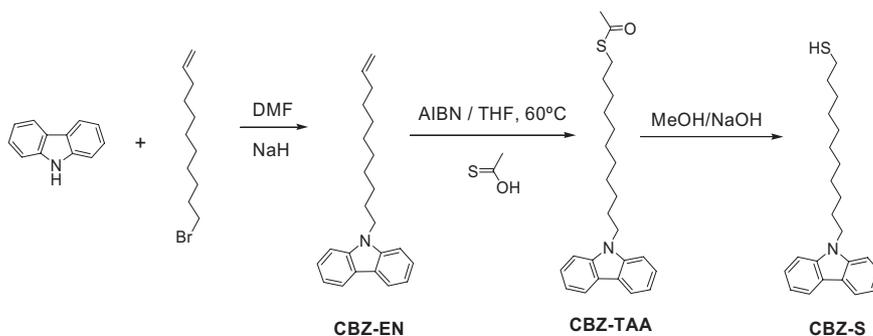
nificant response in the reflectivity change ( $\Delta R$ ), even at very high exposures of up to  $10^{-2}$  M of PMP. For the g-PM-CBZ film, no response was observed upon injecting  $10^{-9}$  M and  $10^{-7}$  M PMP, but a sudden change of  $\Delta R = 0.009$  was observed upon injecting  $10^{-5}$  M (after 30 min) and higher PMP concentrations. A simultaneous response at  $\Delta E = 0.008$  V was observed with potentiometry (30 min; Fig. 3b). The simultaneous changes in  $\Delta R$  and  $\Delta E$  confirmed the adsorption of PMP analyte on the films. A very good signal-to-noise ratio (S/N) was observed. The sensitivity observed with the g-PM-CBZ (detection limit =  $10^{-5}$  M) is due to the free amines available on the dendrimer film and the increased hydrophobicity of the film compared to Au. The free amines are available for H-bonding with PMP, whereas none are present on bare Au. Thus, the polarity of the substrate and presence of the crosslinked film accounts for this increased sensitivity compared with Au. It was also observed that under the present buffer conditions, when the concentration reaches  $10^{-2}$  M, the buffer can no longer maintain its pH and the so-

lution was found to be acidic; therefore, PMP detection was no longer in the desirable physiological conditions. This change in pH can cause a secondary response in the potentiometric signal, degrading the sensitivity of the method.<sup>[18]</sup>

To account for the potentiometric response, one needs to consider the changes in electronic state of the film. The electrochemical crosslinking of the carbazole present on the periphery of the PAMAM dendrimer generates a highly crosslinked, conjugated polycarbazole network (see SI Fig. 2). The potentiometric changes in a polymer film are believed to be due to the formation of charged species such as “polarons” and “bipolarons”, i.e., singly and doubly charged sites in the polycarbazole chain along with their neutralizing counterions.<sup>[10]</sup> Also, the corresponding neutral unoccupied sites that are conformationally fitted to receive the charge and counterions are considered as independent species. However, the nature of the interaction of the reactive species with its local environment is unclear, and if there are several redox processes occurring, then we are dealing with the stationary potential, in analogy with the theory of corrosion processes.<sup>[19]</sup>

In order to enhance the sensitivity it was necessary to match surface polarity, maintain electrochemical activity, and effectively extend the conjugation length of the carbazole. Therefore, the gold substrates were modified by using SAMs of thiol-undec-9*H*-carbazole (Scheme 3). The SAM may also contribute to improved signal transduction based on greater interfacial contact between the Au surface and the electrochemically crosslinked dendrimer through electrochemical grafting. The use of the carbazole-functionalized SAMs for electrochemical grafting has been previously reported by our group.<sup>[10a]</sup>

Both PM-CBZ and Cu-PM-CBZ precursors were used at the same concentration and spin-coated onto SAM-modified Au films to form SM-D and SM-D/Cu<sup>2+</sup> films respectively (Scheme 2). The thicknesses of these dielectric films were found to be ca. 12.4 and 15.2 nm respectively by SPR reflectivity angular scans and AFM profilometry (see SI Fig. 4). The SAM-modified dendrimer films, with and without copper, were then subjected to electrochemical crosslinking. The sensor studies were performed again using 20 mM ammonium acetate buffer (pH 7.2) and 0.1 M NaCl solution. Both reflectivity ( $\Delta R$ ) and potential changes were observed at each interval between injections with varying ( $10^{-9}$  to  $10^{-3}$  M) PMP concentrations, starting at the lowest concentration (Fig. 4a).



**Scheme 3.** Preparation of the thiol-terminated carbazole.

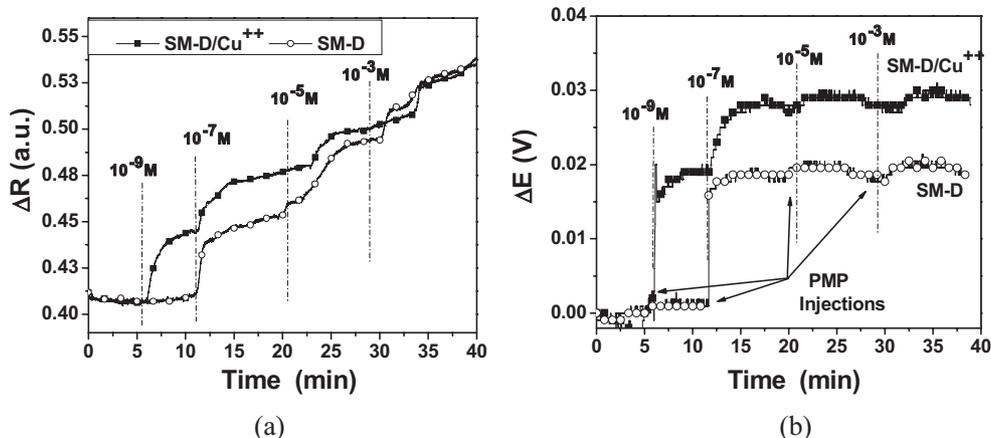


Figure 4. Sensorgram of crosslinked films using a) SPR, b) potentiometry.

A very sharp response (10 s to reach 90 % of signal) at  $10^{-9}$  M (nanomolar) concentration of PMP was observed in the case of SM-D/Cu<sup>2+</sup> ( $\Delta R = 0.035$ ,  $\Delta E = 0.017$  V at 90 % signal), as compared to that of SM-D, which showed response starting only at  $10^{-7}$  M ( $\Delta R = 0.03$ ,  $\Delta E = 0.012$  V at 90 % signal). The SAM-only film (control, not shown) was found to be sensitive only at concentrations above and including  $10^{-3}$  M. Again, very good S/N was observed for all measurements. It is obvious from the results that the higher sensitivity observed in the case of SM-D/Cu<sup>2+</sup> ( $10^{-9}$  M) as compared to that of SM-D ( $10^{-7}$  M) is due to the involvement of Cu<sup>2+</sup> ions, which provides a stronger affinity towards the P=O bond present in PMP. Furthermore, this sensitivity was far superior to the g-PM-CBZ films, i.e., those without the SAMs. These simultaneous changes in both  $\Delta R$  and  $\Delta E$  (Fig. 4a and b) confirm the adsorption of the PMP analyte. Since the SM-D/Cu<sup>2+</sup> films were found to be superior in terms of detecting the lowest concentration and producing the most efficient signals, all further experiments for detection were performed using these films.

The adsorption of the PMP on the ultrathin film of SM-D/Cu<sup>2+</sup> was also confirmed by water contact angle (WCA) measurements. The WCAs of SAM and SM-D/Cu<sup>2+</sup> before exposure to PMP were found to be 65.4° and 67.8°, respectively (Fig. 5a and b). Since the WCA remains almost the same in going from SAM to SM-D/Cu<sup>2+</sup>, this indicates that the SAM generates sufficient polarity (or hydrophobicity), similar to the

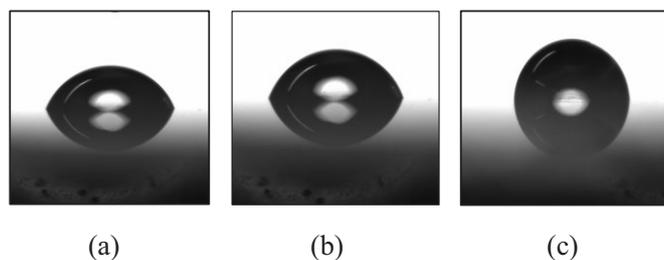
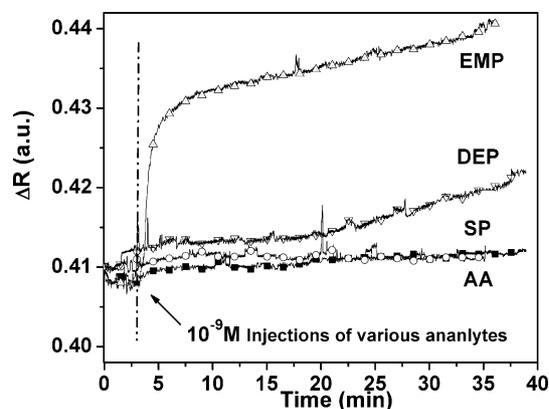


Figure 5. WCA measurement for: a) SAM (SM = 65.4°); b) SM-D/Cu<sup>2+</sup> before exposure to PMP (67.8°); and c) SM-D/Cu<sup>2+</sup> after exposure to PMP (87.5°).

modified dendrimer, to account for non-specific adsorption effects. This accounts for the detection of PMP even at  $10^{-3}$  M for the SAM alone. In contrast, for bare Au, no response was observed (Fig. 3). Therefore, one can reason that the enhancement in sensitivity up to  $10^{-5}$  M for the g-PM-CBZ spin-coated film is due to the fact that the dendrimer can be deposited having an open dendrimer conformation with sufficient polar amine groups present. For the SM-D films, after electrochemical crosslinking on the SAM-modified surface, an increase in interfacial contact between the Au and the dendrimer improves both optical and electrical transduction compared to simple aggregation in the previous case, giving a response of  $10^{-7}$  M. Even better, in the presence of complexed Cu<sup>2+</sup> ions in SM-D/Cu<sup>2+</sup> films, a higher sensitivity of up to  $10^{-9}$  M was observed due to stronger P=O...Cu<sup>2+</sup> ion interactions, as compared to just H-bonding. In this case, the complexation with PMP should reduce the availability of ionic Cu<sup>2+</sup>, i.e., increases the hydrophobicity. Thus, after the exposure of PMP to the SM-D/Cu<sup>2+</sup> films, a change of 87.5° (Fig. 5c) in WCA was observed, confirming the adsorption of PMP. It is reasonable to assume that the increase in WCA may be due to the binding nature of the analyte PMP towards the SM-D/Cu<sup>2+</sup> films, where the hydrophobic *tert*-butyl group is exposed to the film subphase and the active hydroxyl (-OH) and P=O groups are bound to the Cu<sup>2+</sup> ions of the dendrimer film.

In order to further test the selectivity of SM-D/Cu<sup>2+</sup> films, a comparison experiment (Fig. 6) was conducted. This was performed to elucidate whether other P=O bond containing compounds such as ethyl methyl phosphonate (EMP), sodium phosphate (SP), or diethyl phosphate (DEP), and compounds capable of H-bonding such as acetic acid (AA), interfere with the detection of a nerve-agent analog. The structures of all the analytes are shown in Figure 1.

EMP, which is also an analog of toxic nerve agents and analogous in structure to PMP, was also found to show a nanomolar response but the change in reflectivity was almost half of that for PMP. The  $\Delta R$  at 90 % signal was found to be 0.018. Almost no response was obtained for both AA and SP in terms of  $\Delta R$  and  $\Delta E$ . DEP was also found to show a very slow response.



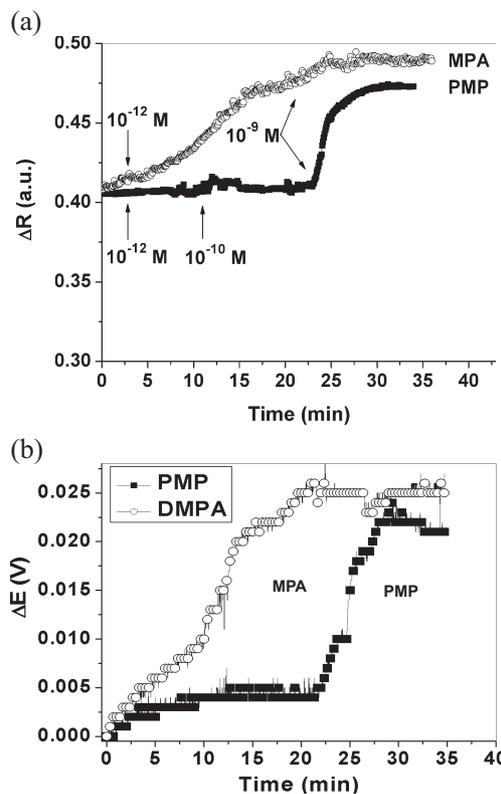
**Figure 6.** SPR sensorogram in buffer for SM-D/Cu<sup>2+</sup> on exposure with various analytes.

The rather slow or lack of response found in the case of analytes other than PMP or EMP clearly suggests that the SM-D/Cu<sup>2+</sup> film are very selective for compounds containing both P=O and hydroxyl (–OH) groups, where Cu<sup>2+</sup> enhances the selectivity by interacting with P=O bond and the remaining (–OH) group can hydrogen-bond with the free primary amines present at the outermost periphery of the dendrimer.

It is well known that organophosphorous compounds are very unstable (Sarin/Soman, a non-persistent class of nerve agent analog, has a half life of 2 min) at higher pH values (close to 10). Moreover, copper ions can further hydrolyze PMP to MPA (a final degradation product of all the organophosphate nerve agents). In order to test the catalyzing effect of copper ions and to test the stability of the PMP in the timeframe of our measurements, a parallel study was conducted using both MPA and PMP starting at picomolar concentration level.

A very slow response was observed with  $\Delta R$  as a function of time when picomolar concentrations of MPA were injected as shown in Figure 7. There was no response observed from SM-D/Cu<sup>2+</sup> film even after injecting PMP at picomolar and decamolar concentration, but once again a very sharp response at nanomolar concentration was observed. The change ( $\Delta R=0.05$ ) was found to be consistent with the previous experiment, indicating a very sensitive and reproducible system. The fact that MPA shows a response starting at the picomolar level could possibly be explained by the fact that it bears an additional hydroxyl group compared to PMP and in principle can be easily tethered to the surface, but at the same time it could require a longer equilibration time.

From Figure 7 it is evident that the changes observed in both reflectivity and response time within the same timeframe for PMP and MPA are very different, suggesting that PMP is being detected in its pristine condition and is not hydrolyzed to MPA. The simultaneous change observed in  $\Delta E$  (Fig. 7b) further supports our results. A higher reflectivity change and a better response time was observed for PMP as compared to that of EMP and MPA, which suggests that the present system is more sensitive and selective towards PMP, although picomolar sensitivity was observed with MPA.



**Figure 7.** a) SPR sensorogram in buffer for SM-D/Cu<sup>2+</sup> using PMP and MPA analytes and b) the corresponding simultaneous potentiometric response.

The fact that the SM-D/Cu<sup>2+</sup> film shows a higher change in  $\Delta R$  and  $\Delta E$  (or sensitivity) and greater selectivity to PMP compared to that of EMP and MPA may be traced to both dendrimer architecture and the presence of Cu<sup>2+</sup> ions. However, this is not easily explained in the absence of modeling or any molecular-imprinting procedures analogous to a crosslinked dendrimer structure.<sup>[20]</sup> Nevertheless the results clearly indicate that both P=O bond and hydroxyl groups are required for an analyte to strongly tether to the crosslinked dendrimer surface. Based on these results we also expect poor sensitivities from other organophosphates and pesticides such as malathion, *tert*-butylphosphonic acid, parathion, etc., in the absence of these chemical structural requirements. This should be an essential criterion for distinguishing false positives in the detection of toxic nerve agents.

### 3. Conclusions

A selective and highly sensitive chemosensor has been reported for nerve agents using SM-D/Cu<sup>2+</sup> films. The adsorption kinetics were monitored simultaneously by using in situ SPR/potentiometric changes at varying concentrations of the analyte. The presence of copper ions in the dendrimer film not only enhances the signal intensity, but also make it selective for PMP. The selectivity was tested against various analytes such

as EMP, AA, DEP, and sodium phosphate. We have also tested the SM-D/Cu<sup>++</sup> film against MPA and compared it with PMP. The MPA shows a poor response time but has sensitivity starting at picomolar concentration levels. It was found that copper ions impart selectivity by binding specifically to P=O and the amines present on the dendrimer increase the number of hydrogen bonds to anchor the nerve-agent analog. The cross-linked films were found to be very stable and in principle can be fabricated easily on portable SPR instruments. Further studies are underway to investigate the actual origin of the potentiometric response, which is believed to be generated because of the reactive species of polycarbazole and its interaction with either the analyte or the dopant ion. Work is underway to develop methods that can be used to sense nerve agents in air.

## 4. Experimental

**Reagents:** G4-PAMAM (10 % w/w in methanol) and all other chemicals used were purchased from Aldrich. All the solvents were distilled and dried properly before use. The buffer solutions were made in MILLI-Q deionized water. The LaSFN9 glass was cleaned by sonicating with 2 % hellmanex solution, washed many times with MILLI-Q water, then washed with ethanol and dried under nitrogen before the gold was vacuum-deposited. The Au/glass (LaSFN9) was plasma cleaned before use.

**Instrumentation:** NMR spectra were taken on a General Electric QE 300 spectrometer (<sup>1</sup>H 300 MHz). UV-vis spectra were recorded using an Agilent 8453 spectrometer. Cyclic voltammetry was performed on an Amel 2049 potentiostat and power lab/4SP system with a three-electrode cell. The SPR setup was based on the Kretschmann configuration using LaSFN9 glass. The Teflon cell was then mounted to the 2-axis goniometer for investigation by SPR. The potentiometric changes were recorded using a Scope view program (Version 1.02) on a Radio Shack digital Multimeter (Cat. No. 22-168A) with a PC data interface attached to a 6115A Precision power supply from Hewlett Packard.

**Synthesis of 4-(2-(9H-carbazol-9-yl)-ethoxy)-benzaldehyde (B-CBZ) (Scheme 1):** A 50 mL solution of 2.05 g (16.8 mmol) 4-hydroxybenzaldehyde, 6 g (21.9 mmol) of CBZ-Br, 1.15 g (4.4 mmol) of 18C6 (Crown ether), and 15.1 g (110 mmol) of K<sub>2</sub>CO<sub>3</sub> in acetone was refluxed for 24 h under nitrogen. After cooling and evaporating the solvent 100 mL water was added the reaction mixture. The mixture was extracted using methylene chloride, and dried with Na<sub>2</sub>SO<sub>4</sub>. After drying and solvent evaporation the mixture was chromatographed using 3:1 methylene chloride/hexane to yield 68 % of the product as white solid. <sup>1</sup>H NMR (δ ppm in CDCl<sub>3</sub>): 9.84 (s, 1H), 8.11 (dd, 2H), 7.73 (dd, 2H), 7.47 (m, 4H), 7.25 (m, 2H), 6.86 (dd, 2H), 4.72 (t, 2H), 4.40 (t, 2H). <sup>13</sup>C NMR (δ ppm in CDCl<sub>3</sub>): 190.8, 166.3, 140.47, 132.08, 131.7, 130.19, 125.9, 120.63, 119.3, 114.6, 108.6, 66.2, 42.15.

**Synthesis of PM-CBZ (Scheme 1):** In a one-necked flask equipped with a 3 Å molecular sieve trap, a solution of 2:1 molar ratio of B-CBZ to PAMAM (128 equivalents based on the 64 terminal primary amine groups) in 30 mL 50 % v/v of methanol and methylene chloride was refluxed for 3 days. The crude product precipitation was performed using 100 mL diethyl ether. The crude product was dissolved in CH<sub>2</sub>Cl<sub>2</sub> and reprecipitated; this procedure was repeated until no starting material was found in NMR. The resulting imine derivative was obtained as beige solid and was found to be 57 % grafted based on 5 % uncertainty of <sup>1</sup>H NMR integrations. However, even after several trials matrix-assisted laser desorption ionization (MALDI) mass spectroscopy failed to show the molecular ion peak. <sup>1</sup>H NMR (δ ppm in CDCl<sub>3</sub>): 8.05–7.97 (b, 134H), 7.47–7.45 (b, 254H), 7.23–7.15 (b, 98H), 6.56 (b, 74H), 4.47–4.4 (b, 72H), 3.84–3.37 (b, 72H), 3.12–2.12 (b, 996H). <sup>13</sup>C NMR (δ ppm in CDCl<sub>3</sub>): 172.5, 160.9, 159.7, 140.9, 132.4, 130.2, 129.3, 126.3, 123.4, 120.8, 119.7, 115.2, 109.2, 66.5, 52.2, 52.0, 51.8, 50.0, 42.8, 37.4, 37.1, 33.8

**Synthesis of Cu-PM-CBZ (Scheme 1):** To a 10 mL vial 18.8 μM solution of PM-CBZ in CHCl<sub>3</sub>/toluene (9:1) was added 2.28 mmol of 2 mL methanol solution of CuCl<sub>2</sub> under vigorous stirring to make the final concentration in a ratio of PM-CBZ/Cu<sup>++</sup> 1:32. The complexation of Cu<sup>++</sup> with PAMAM can be easily seen as there is a change in solution color from light green to yellow after gradual addition of copper chloride solution to the PM-CBZ. The mixture was stirred for 45 min to allow complete complexation.

**Synthesis of 9-(Undec-10-enyl)-9H-carbazole (CBZ-EN) (Scheme 3):** To a stirred solution of 6.0 g (36 mmol) of carbazole in 15 mL dimethylformamide (DMF), NaH 1.83.0 g (39.6 mmol) was added in portions and after complete addition the mixture was heated to 60 °C for 2 h. After the mixture was cooled, a solution of 11-bromoundec-1-ene 9.4 g (40 mmol) in 5 mL DMF was added dropwise to the reaction mixture and was allowed to stir for 48 h at room temperature. The reaction mixture was then poured in water and was extracted using methylene chloride and dried over Na<sub>2</sub>SO<sub>4</sub>. After evaporating the solvent, the crude product was purified by column chromatography using hexane as an eluent gave 9.5 g (82.6 %) of the product. <sup>1</sup>H NMR (δ ppm in CDCl<sub>3</sub>): 8.14 (d, 2H), 7.53–7.42 (m, 4H), 7.27(t, 2H), 5.80 (m, 1H), 4.95 (m, 2H), 4.29 (t, 2H), 2.02 (p, 2H), 1.86 (p, 2H), 1.35–1.22 (m, 12H). <sup>13</sup>C NMR (δ ppm in CDCl<sub>3</sub>): 140.6, 139.3, 125.7, 123.1, 120.3, 118.9, 114.3, 109.9, 43.4, 34.2, 29.8, 29.7, 29.4, 29.3, 29.2, 27.7.

**Synthesis of 11-(9H-Carbazol-9-yl)undecyl Ethanethioate (CBZ-TA) (Scheme 3):** A solution of 9-(undec-10-enyl)-9H-carbazole 1.91 g (6 mmol) in dry tetrahydrofuran (THF; 20 mL) containing thioacetamide 0.63 g (8.32 mmol) and 2,2'-azobis(2-methylpropanitrile) (AIBN) 56 mg (0.172 mmol) was refluxed at 60 °C for 12 h under nitrogen. After cooling the reaction flask 20 mg of AIBN and 0.3 g thioacetic acid was added and refluxed for another 4 h. Concentration of the reaction mixture followed by flash column chromatography (4:1 hexane/CH<sub>2</sub>Cl<sub>2</sub>) gave 2.1 g (88.6 %) of the product. <sup>1</sup>H NMR (δ ppm in CDCl<sub>3</sub>): 8.14 (d, 2H), 7.53–7.42 (m, 4H), 7.27(t, 2H), 4.29(t, 2H), 2.70(t, 2H), 2.35 (s, 3H), 1.92–1.88 (m, 2H), 1.74–1.69 (m, 2H), 1.38–1.26 (m, 14H). <sup>13</sup>C NMR (δ ppm in CDCl<sub>3</sub>): 196, 140.6, 125.7, 123.1, 120.3118.6, 108.9, 43.0, 39.1, 35.1, 32.1, 30.5, 29.4, 29.3, 29.1, 28.9, 27.3.

**Synthesis of 11-(9H-carbazol-9-yl)-undecane-1-thiol (CBZ-S) (Scheme 3):** In a 100 mL round-bottomed flask, CBZ-TA 1 g (2.52 mmol) was dissolved in 10 mL methanol, and CH<sub>2</sub>Cl<sub>2</sub> was added dropwise to make the suspension clear. To this solution 1 mL of 50 wt % NaOH was added under nitrogen and was further allowed to stir overnight. The reaction mixture was neutralized by adding acetic acid. The neutralized solution was then poured into 25 mL water and the organic phase was extracted using methylene chloride. The organic phase was then washed with brine and dried over Na<sub>2</sub>SO<sub>4</sub>. After filtering and concentrating under vacuum, the crude product was further washed with hexane to give the pure product as 0.78 g (87.6 %) of slightly yellowish oil. <sup>1</sup>H NMR (δ ppm in CDCl<sub>3</sub>): 8.14 (d, 2H), 7.53–7.42 (m, 4H), 7.27 (t, 2H), 4.29 (t, 2H), 2.68 (t, 2H), 1.92–1.88 (m, 2H), 1.74–1.69 (m, 2H), 1.38–1.26 (m, 15H). <sup>13</sup>C NMR: δ (ppm) 140.6, 125.7, 123.1, 120.3118.6, 108.9, 43.0, 39.1, 35.1, 32.1, 29.3, 29.1, 29.0, 28.9, 28.4, 27.3.

**Synthesis of Carbazole-Terminated SAMs (CBZ-S/Au) (Scheme 3):** The SAM was formed on a plasma cleaned Au/LASFN9 glass film. The Au film was dipped into a 1 mM solution of 11-(9H-carbazol-9-yl)-undecane-1-thiol in absolute ethanol/THF (9:1). The film was then rinsed with anhydrous methylene chloride and absolute ethanol, dried under nitrogen and stored in a vacuum desiccator for further use.

**Synthesis of Crosslinked SM-D/SM-D/Cu<sup>++</sup>/SM (Scheme 2):** Both PM-CBZ and Cu-PM-CBZ precursors were taken in the same concentration (15.7 μM) in chloroform: toluene: methanol (9:1:2 v/v) ratio and then spin-coated on (2.5 cm × 2 cm) bare gold and SAM-modified gold films (Scheme 3). The electrochemical crosslinking was then performed in a three electrode cell 0.1 M tetrabutylammonium hexafluorophosphate (TBAHFP) as supporting electrolyte in 15 mL acetonitrile using cyclic voltammetry (see Supporting Information).

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