รายงานวิจัยฉบับสมบูรณ์

# การใช้ LbL modification ด้วย PEG และ PEI บน PE เพื่อตรวจจับตะกั่ว

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สัญญาเลขที่ TRG5680076

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัยและ
วิทยาลัยนานาชาติ มหาวิทยาลัยมหิดล

# บทคัดย่อ

**รหัสโครงการ :** TRG5680076

**ชื่อโครงการ :** การใช้ LbL modification ด้วย PEG และ PEI บน PE เพื่อตรวจจับตะกั่ว

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การเจือปนของตะกั่วเป็นปัญหาใหญ่ ในประเทศที่เจริญแล้วและประเทศที่กำลังพัฒนา การเจือปนของสารตะกั่วพบได้ในของเล่นเด็กสีทาบ้านและอาหารเพื่อที่จะแก้และบรรเทาปัญหาเหล่านี้พวกเราได้ใช้เทคนิคการประกอบเลเยอร์บายเลเยอร์เพื่อการดัดแปลงพื้นผิวของโพลีเอทิลีนฟิล์มมาเป็นอุปกรณ์ในการตรวจจับตะกั่วราคาถูกกลุ่มการค้นคว้าของเราได้นำโพลีเอทิลีนมาจากขวดนมที่ใช้แล้วเพื่อการใช้ขยะมานำใช้ใหม่และลดต้นทุนเริ่มโดยการเปลี่ยนผิวของโพลีเอทอลีนให้เป็น carboxylic acid โดยกรดซัลฟูริคและโครเมี่ยมไตรออกไซค์แล้วต่อด้วยโพลีเอทีลีนไกลคอลหรือโพลีเอทิลีนอิมมีนเพียงชั้นเดียว และชุบด้วยโซเดียมโรไดโซเนต หรือไดไตโซนซึ่งเป็นสารตรวจจับตะกั่วผลปรากฏว่าสามารถตรวจจับตะกั่วได้เพียงแค่ระดับ 5000 ppm เท่านั้นเราจึงได้ใช้การสร้างพันธะสองชั้นด้วยโพลีเอทิลีนอิมมีนก่อนและตามด้วยโพลีเอทีลีนไกลคอลแล้วชุบพื้นผิวด้วยโซเดียมซัลฟาย ผลปรากฏว่าสามารถตรวจจับตะกั่วได้ต่ำถึง 50 ppm โดยการเปลี่ยนสีของพื้นผิวจากขาวเป็นสีน้ำตาลหรือดำขึ้นอยู่กับความเข้มข้นของตะกั่วโพลีเอทิลีนฟิล์มที่ได้ผ่านกระบวนการนี้สามารถนำมาใช้จับตะกั่วซ้ำได้ประมาณห้าครั้งและสามารถเก็บได้ถึงสามอาทิตย์เป็นอย่างต่ำ

**คำหลัก :** การประกอบเลเยอร์บายเลเยอร์ โพลีเอทิลีน ตะกั่ว โลหะหนัก

# Abstract

**Project Code :** TRG5680076

**Project Title :** Surface Modification of Polyethylene Film by Covalent Layer-by-Layer Assembly using Polyethyleneglycol and Polyethylene imine, as a Tool to Detect Lead (Pb) Contaminants

**Investigator :** Chayanant Hongfa

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**Project Period :** June, 2013 to August, 2015

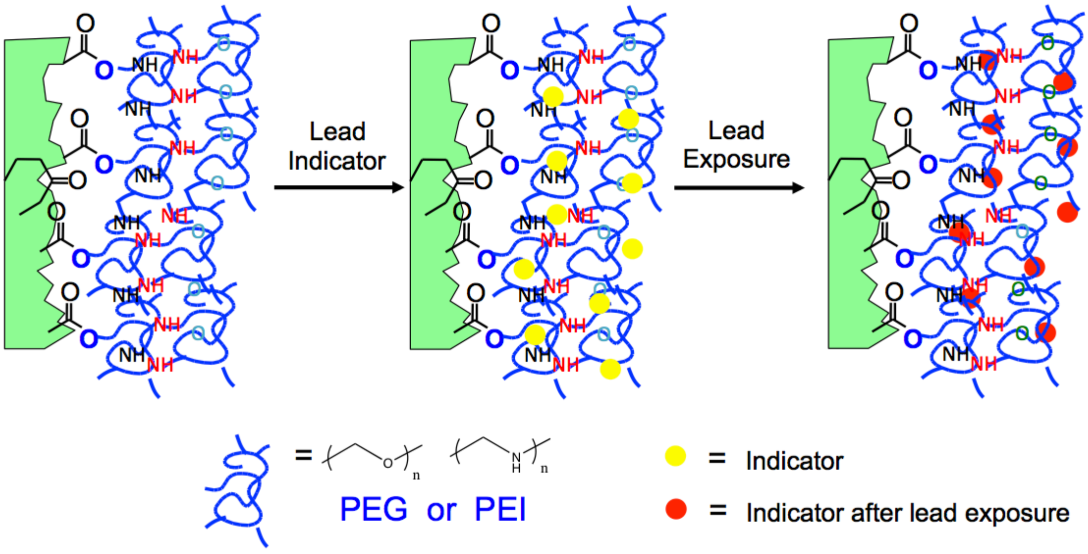
Lead (Pb) contamination is a major problem in both developed and developing countries. The contaminations are often found in products such as children toys, paints, and food products. As a result, there is an urgent need for an economical heavy metal indicator. In order to address this issue, the modern approach of layer-by-layer (LbL) assembly to modify a surface of polyethylene can be used. The strip of polyethylene film (PE) was obtained from used water and milk bottles. A solution of chromium trioxide and sulfuric acid was then used to oxidize the surface of PE into multiple carboxylic acid groups. First the monolayer modified with polyethylene glycol (PEG) to form PE-PEG and with polyethylene imine (PEI) to form PE-PEI were synthesized and embeded with sodium rhodizonate and dithizone as lead detecting dyes. The sensitivity of the monolayer modified PE strips was too low and could only detect the present of lead as low as 5000 ppm. The bilayer modified PE strip with polyethylene glycol and polyethylene imine through the process of the amidation followed by the nucleophilic substitution, yielded much better result with the sensitivity as low as 50 ppm. The newly made PE with hydrophilic surface was non-covalently embedded with sodium sulfide as a detecting dye that turned light brown or black in the present of lead. The sodium sulfide coated bilayer PE-PEI-PEG strip could be reused up to five times and was shelf stable for longer than three weeks.

**Keywords :** Layer by Layer Assembly, Polyethylene, lead, and Heavy Metal

## 1. Introduction

Lead has been used commonly in household appliances since its discovery that it is naturally abundant in the earth’s crust. Lead’s properties of being a very soft and malleable metal in addition with being resistant against corrosions, making it very useful in pottery, paint, and utensils.[1] Many of the traditional herbal medicines are also known to contain lead especially in Thailand, a recent survey of Thai children in the Bangkok area were found to have the average blood lead concentration to be 5.65 mcg/dL, with 8.1% of children having lead levels greater than 10 mcg/dL.[1] The United States Centers of Disease Control and Prevention (CDC) has set a regulating standard for lead to be no more than 10 mcg/dL. Therefore lead poisoning poses severe heavy metal poisoning hazards especially in Thailand.

The current existing lead detectors have good lead detecting mechanisms, but they are expensive. Therefore, there is a need for a lead detector that is cheap and easy to handle by the general population to measure lead. In order to address this issue, the modern approaches of layer-by-layer (LbL) assembly to modify a surface of polyethylene film, was utilized. LbL assembly is a well-known technique in covalently or ionically embedding various molecules to the surface of the polymer.[2-8] The novelty of this research lies upon the usage of cheap starting material and the new approach, which is relying upon the entanglement of lead detector molecules upon the surface in the manner similar to how Velcro® hook-and-loop system as shown Scheme 1. This non-covalent approach is an extension of the work by Berbgbreiter and Grunlan et al., which utilized hyperbranched polyethylenen imine on the surface of polyethylene film to increase the level of carbon black embedment onto its surface.[5]



Scheme 1: The entanglement of lead detectors on the surface of a modified polyethylene film

## 2. Experimental Procedure

### 2.1 Material and Characterization

All chemicals including PEI ($M_n$ = 800) and PEG ($M_n$ = 4000) were purchased from Sigma-Aldrich and used as received. The PE strips were obtained from emptied milk bottles of Farm Chokchai Umm!..Milk®. All of the characterizations were done by Perkin Elmer FT-IR Spectrometer 100 with an ATR attachment. The contact angle measurements were done by Tensiometer model TL-100.

### 2.2 Oxidation of Polyethylene (PE-COOH)

The emptied milk bottle was thoroughly cleaned and cut into small rectangular strips of roughly 3 cm x 1.5 cm. These polyethylene strips were shaken in the mixture of $CrO_3/H_2O/H_2SO_4$ (1:2:1 by weight) for three hours at 90°C. After the oxidation was finished, the polyethylene strips were rinsed with water and then acetone and allowed to air dry.

### 2.3 Preparation of Polyethylene Glycol Grafts on Oxidized PE Surface (PE-PEG)

PE-COOH strips were shaken in a solution of toluene (4 mL) with 1 mL of oxalyl chloride and 2 drops of DMF and then refluxed for 1 hour. The PE-strips were wiped dried and then immersed in a mixture of 6 g of PEG, 0.4 mL of pyridine, and 7 mL of methylene chloride for 12 hours.

### 2.4 Preparation of Polyethylene imine Grafts on Oxidized PE Surface (PE-PEI)

PE-COOH strips were shaken for 3 hours at 100°C with a solution of toluene (20 mL) that contained 0.5 g of boric acid and 2 g of $PEI_{800}$. After the reaction was finished, the newly form PE-PEI strips were rinsed with water and then acetone and allowed to air dry.

### 2.5 Preparation of Polyethylene Glycol Grafts on PE-PEI (PE-PEI-PEG)

PE-PEI strips were shaken with 1 g of Tos-$PEG_{4000}$-Tos in 7 mL of methylene chloride for 12 hours. After the reaction was finished, the newly form PE-PEI-PEG strips were rinsed with water and then acetone and allowed to air dry.

### 2.6 Entrapment of Pb indicators

Sodium rhodizonate and sodium sulfide were entrapped onto the surface of PE-PEI-PEG. A saturated aqueous mixture containing each of the indicators was prepared and then strips of chosen modified PE were immersed into the solution of choice (sodium rhodizonate, dithizone, or $Na_2S$) for 2 hours at room temperature. After the entrapment was complete, the coated modified PE was wiped dried of any excess aqueous solution.

## 3. Results and Discussion

### 3.1 Oxidation of Polyethylene (PE-COOH)

To create a surface suitable to LbL assembly, PE strip was oxidized by Jones reagent to form PE-COOH as shown in figure 1. The newly formed PE-COOH was scanned by ATR-IR as shown in Figure 2. The left hand side was the IR spectrum of an unmodified PE strip and the on the right hand side was the IR spectrum. The newly formed PE-COOH contained a new C=O peak of carboxyl group at 1720 $cm^{-1}$, which confirmed the formation of PE-COOH. The OH peak was not present, but the data was consistent with the work by Bergbreiter et al.[2a] The contact angle of a PE-COOH strip was 87.4° as shown in Figure 3.

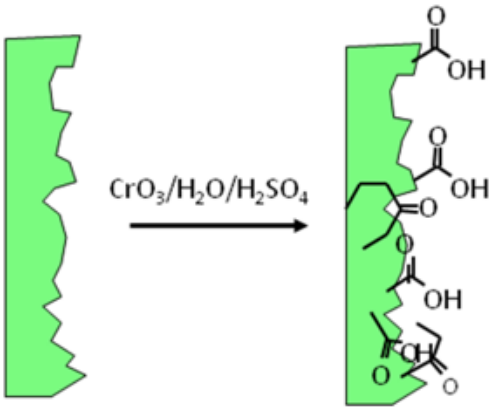


Figure 1: Formation of PE-COOH from PE strip

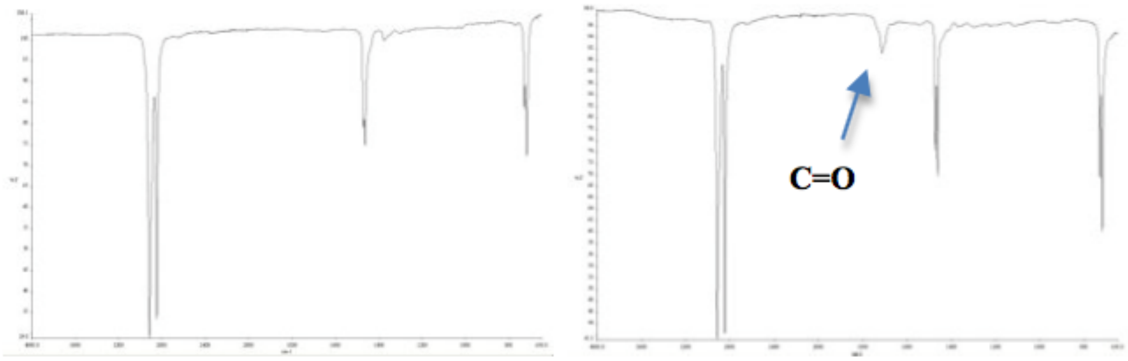


Figure 2: FT-IR spectra of PE strip on the left hand side and PE-COOH on the right hand side

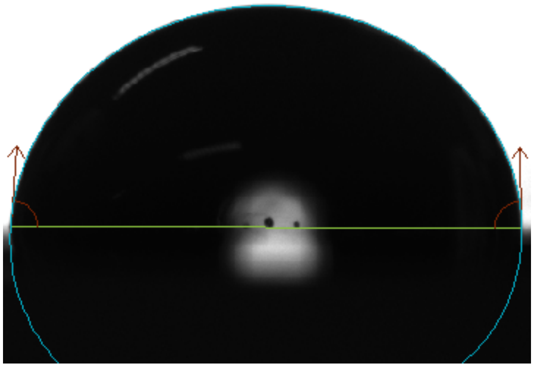


Figure 3: The contact angle measurement of PE-COOH

### 3.2 PEG Grafted on Oxidized PE Surface (PE-PEG)

The carboxylic acid group of COOH was allowed to form an ester group with $PEG_{6000}$ with hydroxyl end groups as shown in Figure 4. The newly formed PE-PEG was scanned by ATR-IR to confirm the attachment of PEG onto the surface of oxidized PE strips. The new IR peaks around the area 1050-1200 $cm^{-1}$ showed the present of newly attached C-O peaks from PEG as shown in Figure 5. The contact angle of a PE-PEG strip was $67^{\circ}$ as shown in Figure 6. The decrease in a contact angle from $87.4^{\circ}$ to $67^{\circ}$ confirmed a decrease in the hydrophobicity of the surface due to the newly attached PEG, which is hydrophilic in nature.

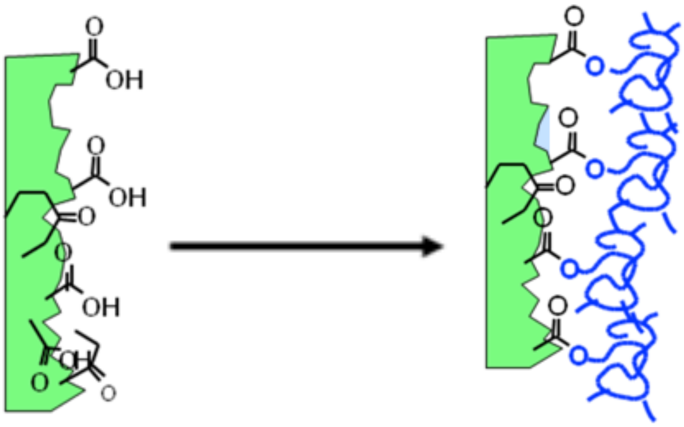


Figure 4. Formation of PE-PEG

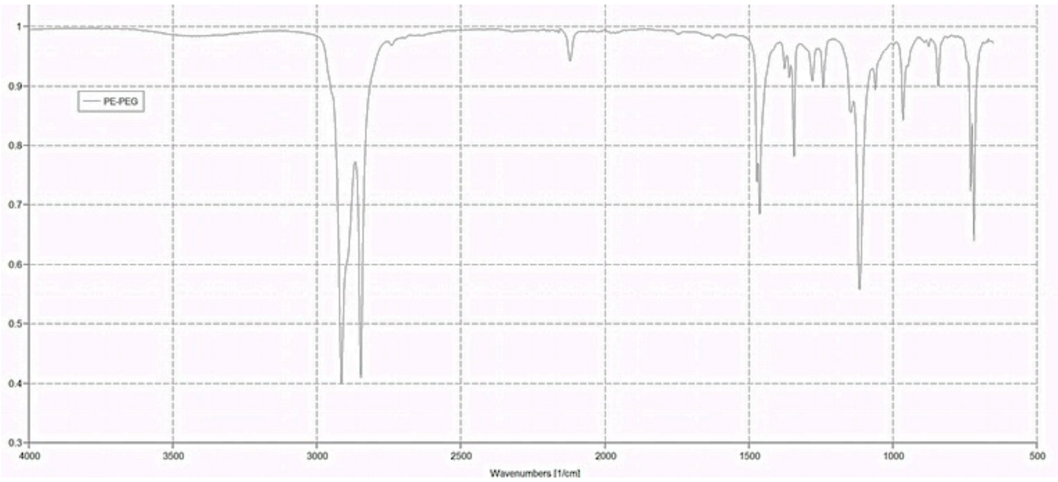


Figure 5. The IR spectrum of PE-COOH

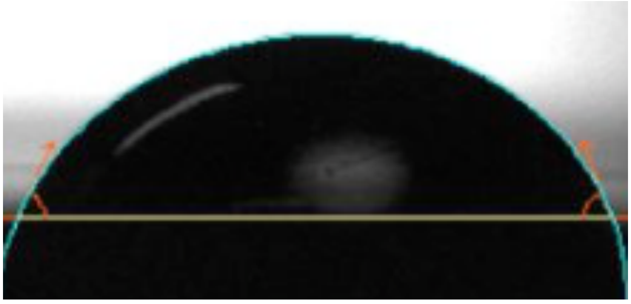


Figure 6: The contact angle measurement of PE-PEG

### 3.3 PEI Grafted on Oxidized PE Surface (PE-PEI)

The attachment of PEI to the surface of PE-COOH was achieved in the amidation of the PE-COOH with $PEI_{800}$ as shown in figure 7. The newly formed PE-PEI was scanned by ATR-IR to confirm the attachment of PEI onto the surface of oxidized PE strips. The new N-H stretching frequency around the area 3300-3500 $cm^{-1}$ and C=O peak of amide around 1610-1630 $cm^{-1}$ showed the present of newly attached PEI as shown in Figure 8. The contact angle of a PE-PEI strip was $71^{\circ}$ as shown in Figure 9. The decrease in a contact angle from $87.4^{\circ}$ to $71^{\circ}$ confirmed a decrease in the hydrophobicity of the surface due to the newly attached PEI, which is hydrophilic in nature.

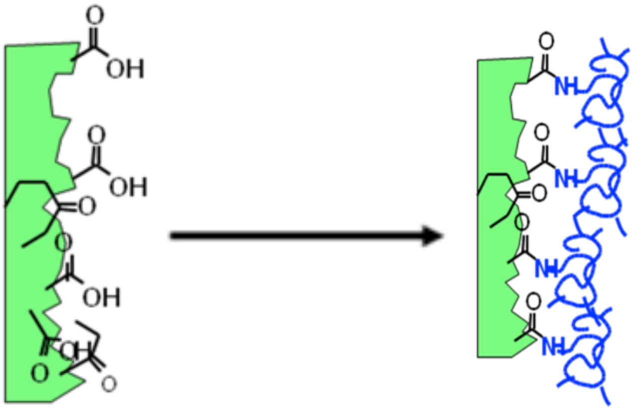


Figure 7. Formation of PE-PEG

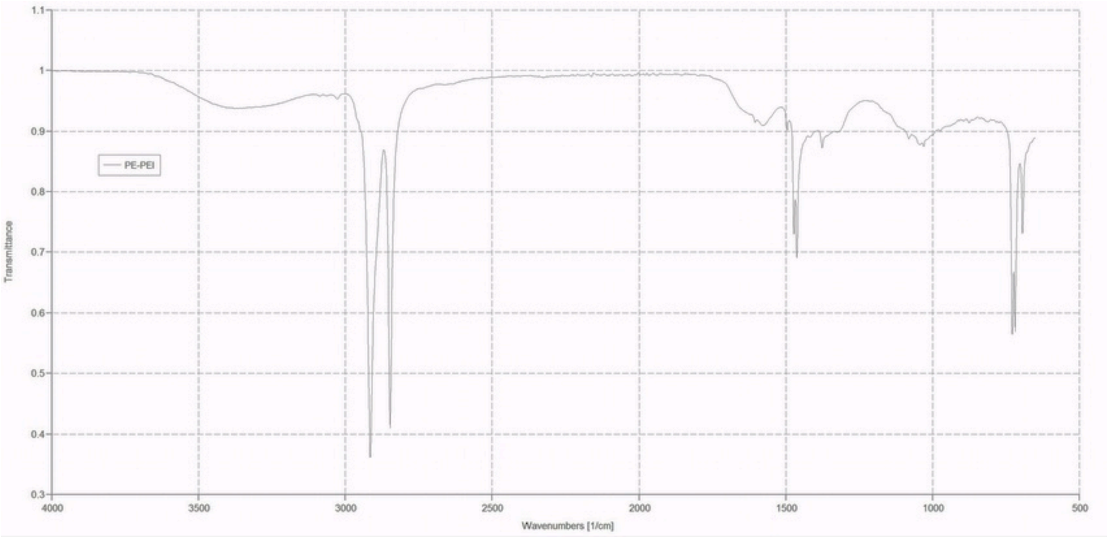


Figure 8. The IR spectrum of PE-PEI

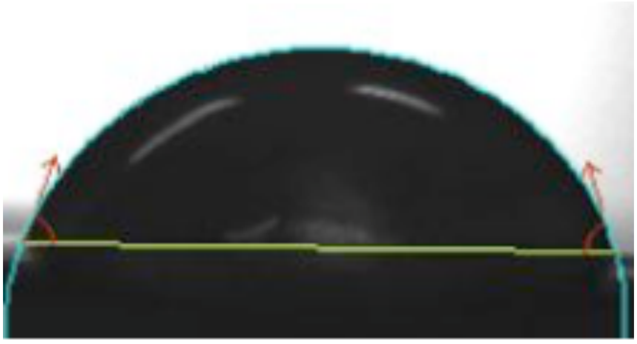
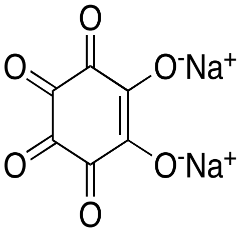


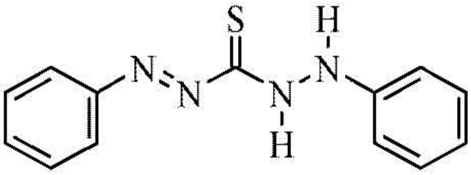
Figure 9: The contact angle measurement of PE-PEI

### 3.4 The Entrapment of Pb Indicators onto the Surface of PE-PEG and PE-PEI

In order to turn modified PE strips into lead indicators, we non-covalently embedded two different types of known lead indicators. They were sodium rhodizonate and dithizone. First, we tested sodium rhodizonate and the result is shown in Figure 10. The unmodified PE strip did not have any visible embedment. Both the PE-COOH and PE-PEG strips also did not show any significant level of embedment. However PE-PEI surface showed high level of embedment of sodium rhodizonate (orange color).



Sodium Rhodizonate



Dithizone

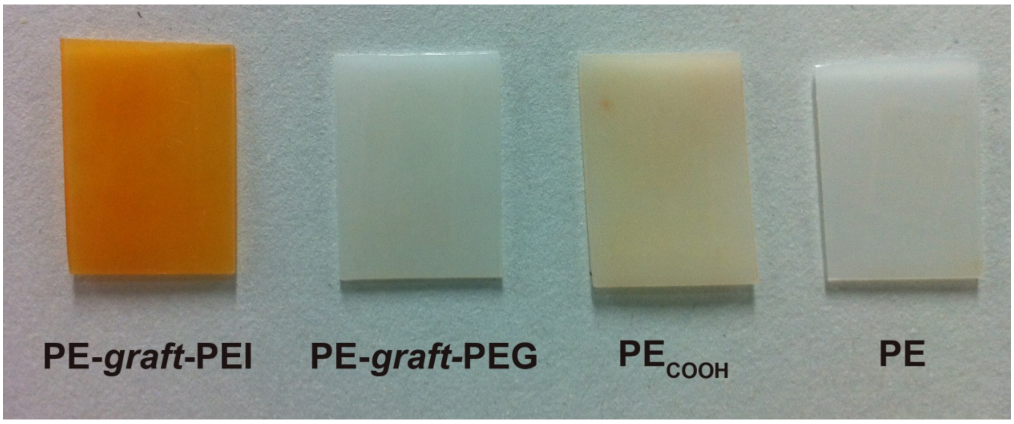


Figure 10: The embedment of sodium rhodizonate onto various types of PE strips

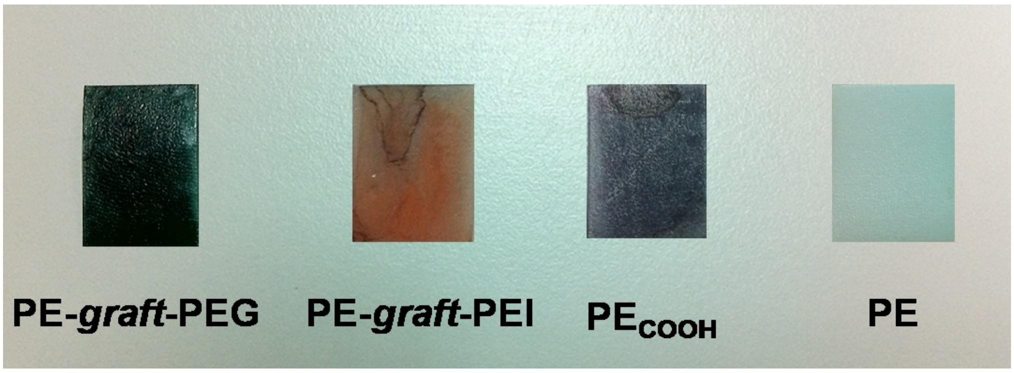


Figure 11: The embedment dithizone onto various types of PE strips

The result of the embedment of dithizone was the opposite of sodium rhodizonate. The PE-PEG showed a high level of dithizone (dark bluecolor) embedment on its surface. As for PE-PEI, there was a change in color due to an unknown interaction of dithizone and PEI on its surface, which turned the strip red, rendered the strip unusable for the detection of lead. The unmodified PE surface did not show high level of the embedment. As for the PE-COOH, the embedment was also insignificant.

### 3.5 Lead Testing of Monolayer Modified PE

Only two combinations of modified PE surfaces and indicators embedment were successful. They were the PE-PEI/sodium rhodizonate and PE-PEG/dithizone combinations. The colorized PE-PEI strips by sodium rhodizonate were exposed to lead and a color change of yellow to pink occurred as shown in Figure 11. The solution of lead (II) nitrate with varying concentration from saturated, 10,000 ppm, and 5,000 ppm were tested and the result is shown in Figure 11. The change in color from orange to red was intense in the case of a saturated solution, but significantly reduced in 10,000 ppm and nearly invisible in 5,000 ppm. Therefore, this particular embedment technique is not very practical.

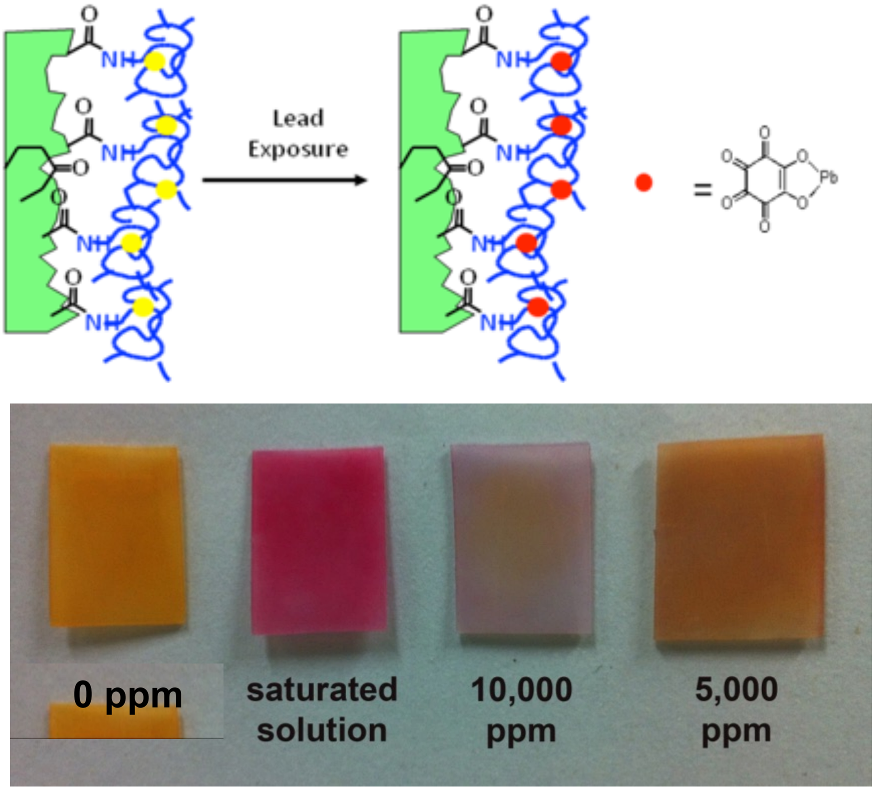


Figure 11: The lead exposure test of PE-PEI/sodium rhodizonate

As for the case of PE-PEG/dithizone combinations, the result is shown in figure 12. The colorized PE-PEG strips by dithizone were exposed to lead and a color changed from dark blue to pink occurred as shown in Figure 12. The solution of lead (II) nitrate with varying concentration from saturated, 5,000 ppm, 500, and 50 ppm were tested and the result is shown in Figure 11. The detection level was sensitive enough, but we discovered that the colorized PE-PEG/dithizone combination turned into pink without an exposure to lead solution within 2 hours, rendering this combination unpractical.

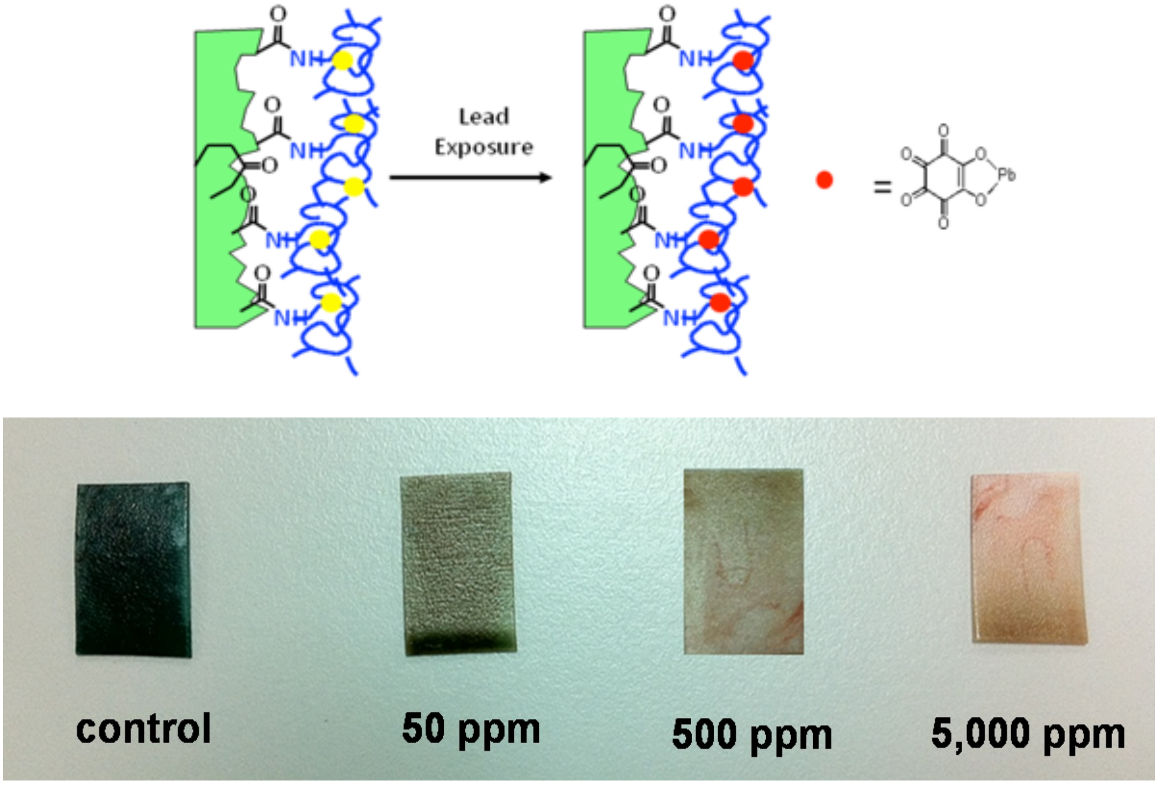
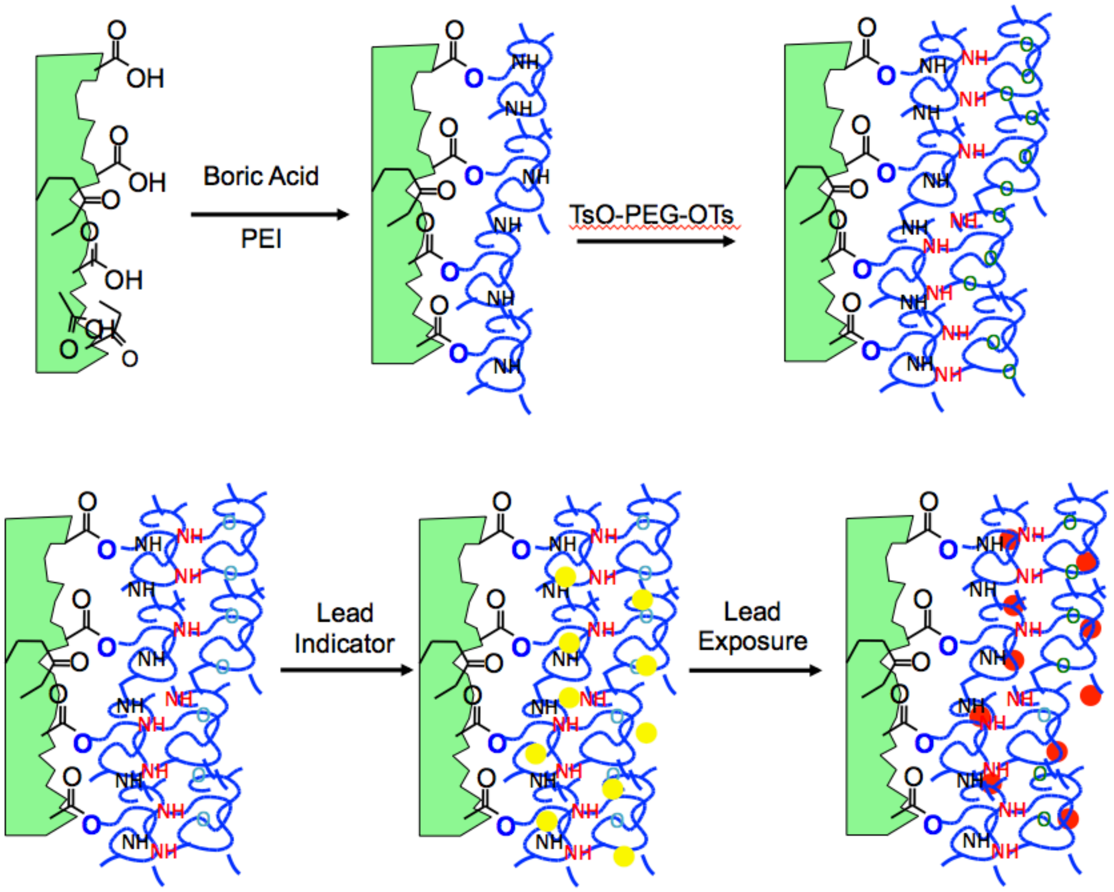


Figure 12: The lead exposure test of PE-PEG/dithizone

### 3.6 Bilayer Modification of PE Surface (PE-PEI-PEG)

The approach to the bilayer modification of the PE surface is similar to that of the monolayer PE-PEG and PE-PEI, but the first set of polymer must be PEI, then follow with the treatment of the terminally modified PEG with tosylate group (TsO-PEG-OTs) as shown in Scheme 2. This approach added an additional layer of polymer and provided more room for the non-covalent embedment of a lead indicator. Since the result from the monolayer proofed that sodium rhodizonate and dithizone did not function well as an indicator for our non-covalent embedment, we decided to use sodium sulfide ($Na_2S$) as an indicator for the bilayer PE-PEI-PEG.



Scheme 2: The bilayer assembly of PE-PEI-PEG and the dye embedment

The procedure from PE-COOH to PE-PEI was modified to use boric acid because it was a cheaper alternative and yield the same result with much shorter time of three hours instead of thirteen hours by the oxalyl chloride route. The addition of PEG was completed with a FT-IR spectrum essentially containing IR peaks from the combination of PEG and PEI into one spectrum as shown in Figure 13. The contact angle of PE-PEI-PEG was 77$^{o}$ as shown in Figure 14. The new N-H stretching frequency around the area 3300-3500 cm$^{-1}$, C=O peak of amide around 1610-1630 cm$^{-1}$, and C-O peaks of PEG from around 1050 – 1200 cm$^{-1}$ confirmed the present of both PEI and PEG on the modified PE.

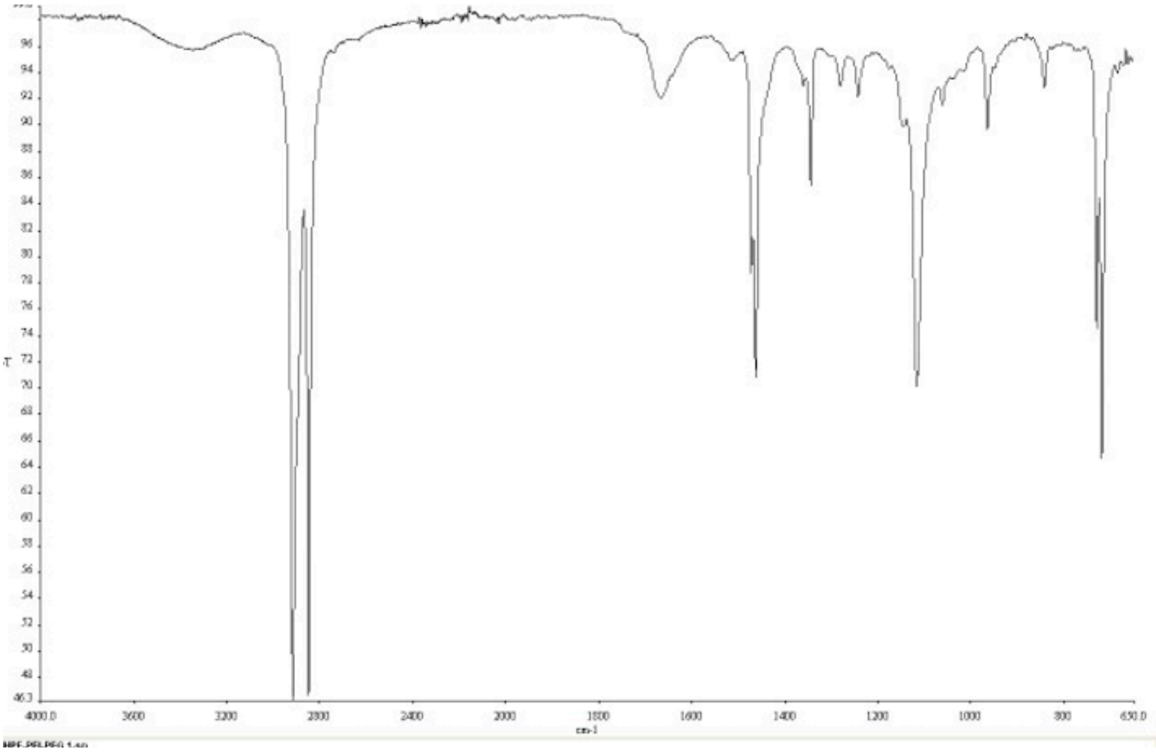


Figure 13. The IR spectrum of PE-PEI-PEG

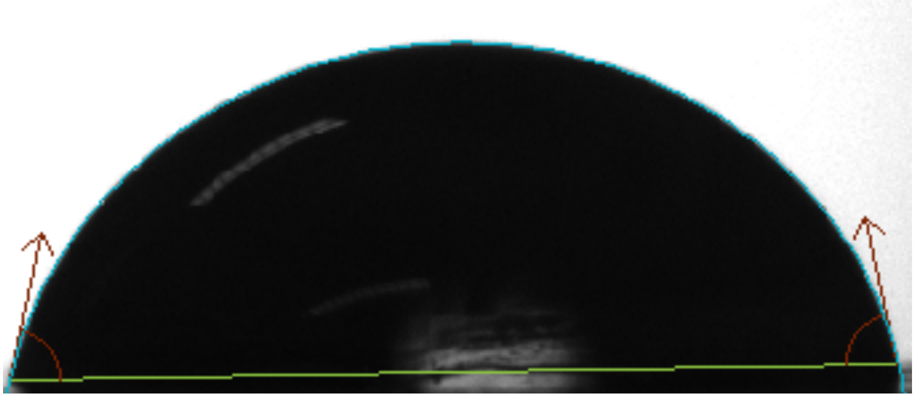


Figure 14: The contact angle measurement of PE-PEI-PEG

### 3.5 Lead Testing of Bilayer Modified PE

Sodium sulfide was chosen as the indicator of choice because it was widely utilized as a lead indicator in an instant lead indicator such as Abotex® Lead Inspector (Figure 15), the kit was easy to use but the price is very expensive at $12.99 for one kit with four usages. First the PE-PEI-PEG strips were submerged into a saturate solution of sodium sulfide for two hours and then wiped dried. The sodium sulfide embedded bilayer modified PE was then tested with 50 ppm, 500 ppm, and 5000 ppm solution of lead (II) nitrate. The result is shown in Figure 16.

Figure 15: Abotex® Lead Inspector

**PE-COOH** **0 ppm** **50 ppm** **500 ppm** **5000 ppm**

Figure 16: The lead exposure tests of PE-PEI-PEG

The PE-COOH was tested as a control and tested with 5000 ppm solution of lead (II) nitrate without any visible change in color. The same can be said for the 0 ppm. The lowest visible change in color to light brown was visible in 50 ppm solution, the color is a lot more visible at 500 ppm, and eventually turned black for 5000 ppm. The minimum detectable level was determined to be 50 ppm. The droplet of lead solution can be wiped dried and reused up to 7 times. The sodium sulfide embedded PE-PEI-PEG was left dried in a close container for 3 weeks and tested again for functionality and the result remained the same.

## 4. Conclusion

The effects of both monolayer modified PE strips (PE-PEG and PE-PEI) were compared with the non-modified and the bilayer modified PE-PEI-PEG strip. In the absence of mono- and bilayer surface modification, all three indicators sodium rhodizonate, dithizone, and sodium sulfide did not embed onto the surface at all. Out of these three dyes, sodium sulfide was the most sensitive and stable dyes for the coating of the bilayer modified PE strips. Grafting the second layer of PEG onto the surface of PE-PEI provided excellent non-covalent embedment of lead detecting dyes and created a field sensitive lead testing strips as low as 50 ppm. This sodium sulfide coated bilayer PE-PEI-PEG added the reusability and robustness into a field lead (Pb) testing kit. The PE-PEI-PEG lead testing strip could be recycled up to seven times and was shelf stable for more than three weeks. These initial results demonstrated the ability to use LbL assembly on a surface that is traditional not traditionally coatable with water-soluble dyes. Future work will seek to improve the adhesion of the non-covalent embedment of dyes and also evaluate more kinds of indicators to detect other type of materials.

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