Urease Enzyme Biosensor for Water Pollution Detection

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ABSTRACT

Urea is the most containing nitrogen substance in the Urine of mammals. It plays an important role in the metabolism of nitrogen containing compounds by animals and human. A Biosensor for Urea was made by immobilizing Urease enzyme and Bromo stain which both obtained from sigma- Aldrich company on the microparticales surface were prepared through encapsulation successively by LBL self- assembly process of polyelectrolyte used PASS & PAH Polymers of eight layers. The size of nanoparticales was (5.05 – 5.87 nm). In other case some of these microparticales were removed there cores by HCL acid and filled with Bromo stain which is sensitive to change in PH. The microparticales were prepared and determined there size, layers under the SEM microscopy. The temperature, PH buffer concentration, Urease enzyme concentration, and enzyme immobilizing procedure were investigated. Microparticales prepared with Urease enzyme gives high sensitive response time 1-2 min. This Biosensor can use to determine the pollution in water and these microparticales made possible Biosensor to carry inside many medical materials in the future.

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1. Introduction

Urea is the main compound in the urine of the mammalian and the human. Its colorless, highly soluble in water and the alcohol, solid, and almost non-toxic (LD_{50} is 15 g/kg for rats)[1].It's found in the blood in most of the mammalian. In the Urea cycle, the liver forms it from most nitrogen excretion by combining two ammonia molecules (NH3) with a carbon dioxide (CO2) molecule. Urea travels from the liver to the kidneys and is excreted from the body with in urine. Almost half of the solids found in urine are urea or produced from amino acids that not used for the synthesis of proteins[2]. Urea is an organic compound and its chemical formula $CO(NH_2)_2$ [3]. In case the wastewater was throwing in rivers Urea can increase, the algal blooms and some of these blooms can produce toxins, and when it found in the runoff from fertilized land this increase of algal blooms. Urease biosensors for detection of urea consisting of microparticales covered by PH sensitive dye. This Biosensor could be used for general water pollution with Urea. [4] Urease enzyme plays a role in our body catalyses and control most biochemical reactions. [5] the enzyme Urease from Canavalia ensiformis (Jack bean) powder, breaks down urea to ammonia and carbon dioxide:

$$CO(NH_2)_2 + H_2O \rightarrow 2\,NH_3 + CO_2 \qquad \ldots.1$$

Urease is an enzyme that catalyzes the hydrolysis of urea, which forming ammonia and carbon dioxide. This enzyme is found in large quantities in soybeans, jack beans and seeds of other plants. The possibility of used a PH indicator dyes to detect Ammonia which its solution has a high PH nearly 9.2. Biosensors development is still needs of fast and On-site analysis with little pre-processing. [6] Several sensors, such as whole-cell and enzyme-based biosensors, have been defined as rapidly detector of urea.

2. Experimental

Urease enzyme is from Canavalia ensiformis (Jack bean) powder, Other chemicals used are: (Polyalylamine Hydrochloride (PAH), Sodium Polystyrene Sulfonate-PSS, Tris-HCl , PBS buffer, Urea and Bromo-Staine-bromothymol blue, (all from Sigma-Aldrich).

2.1 Capsules preparation:

Preparation of polyelectrolyte microparticales from MgCO3 core particles by consecutive coating with Layers of PAH (Polyalylamine Hydrochloride) and PSS (Polystyrene Sulfonate

Sodium Salt) eight layers. Such particle may have an inclusion of organic pH sensitive dye either in the polyelectrolyte shell or in the core (by removing the core material in acid and substituting with dye). The particles produced can be further modified with Urease enzyme on the shell of the microparticales [7] by consecutive coating of inorganic $CaCO_3$ or $MgCO_3$ template particles with PSS/PAH layers and dissolving the core at low pH. Scanning Electron Microscope (SEM) was used to view micro-particles at different stages of their fabrication and functionalization (Fig. 1).

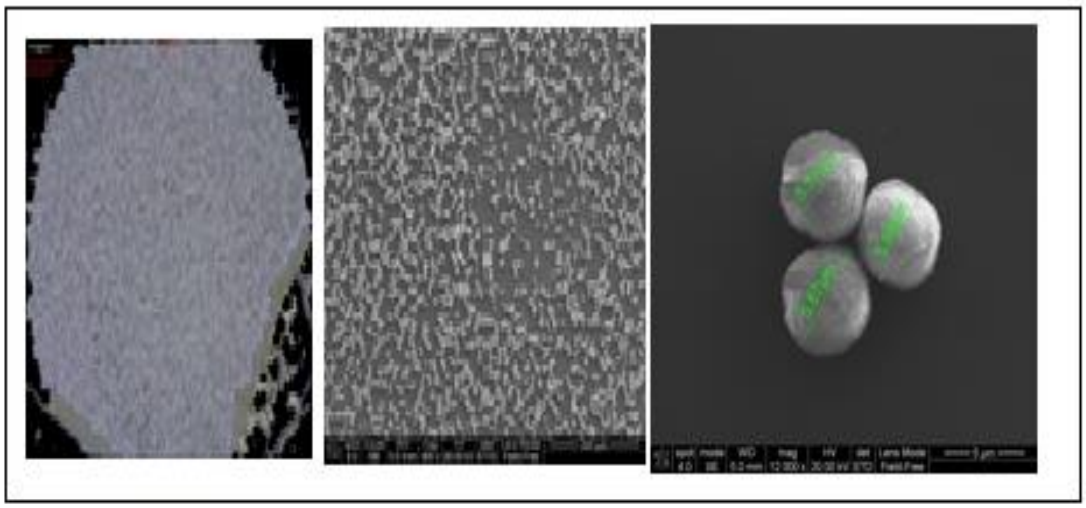

Figure 1: Microcapsules fabricated by LbL technique under SEM microscopy.

Prepared polyelectrolyte microparticles by coating inorganic $MgCO_3$ template particles with layers of polyanions (sodium poly-stryrene sulfonate or PSS) and polycations (polyallylamine hydrochloride PAH). Fine particales have been obtained these micro-particles were functionalized with Bromo stain and Urease enzyme. During LbL assembly, oppositely charged polymers are electrically bonded to each other to produce multiple thick insoluble layers. The thickness of this multilayer film is controlled by 2-3 nm number of precipitated multi-electrolyte bilayer. Then by slowly alternating the adsorption of polycations and polyanions, microcapsules are form with a size of 5-5.8 nm that are exact to within a few nanometers can be formed [8]. In adding the structure of the microcapsules can polyelectrolytes structure by natural materials such as polysaccharides, enzymes and proteins. Even charged nanoparticles will form LbL coatings and shells. The only condition is that the positive and negative components are alternated [9]. This means that the relatively simple nanoplating process has developed into a powerful platform for modifying the surfaces of materials or encapsulating different substrates (Fig.1).

Therefore, since the 1994 more than ten thousand papers have been published telling various aspects and applications of the LbL method [10].

2.2.Prepare solutions:

Suspension of Urease enzyme: 10 mg of urease enzyme in 10 ml of water. The enzyme urease breaks down Urea into ammonia and carbon dioxide. Ammonia (NH3) solution has a high pH which can be detected using a simple pH indicator like Bromo phenol stain which change in color from yellow to blue when the pH is change from acidity to alkaline. Urease enzyme at PH 4.0 be as a polycation at 25C0 temperature, but in PH 8.0 be as polyanion.

Urea solution: dissolved 60mg of Urea in 50ml of water.

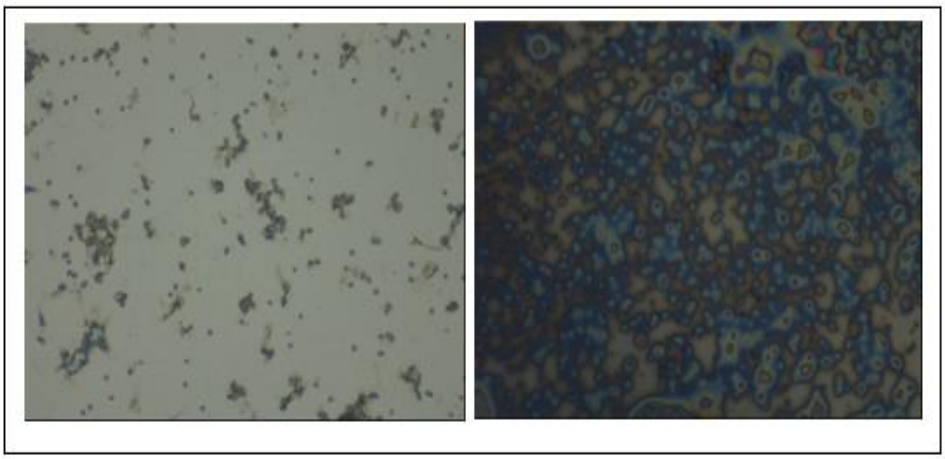

Figure 2: Capsules with Bromo stain at different PH under the AFM.

3. Results and discussion

Following the procedure developed earlier [11] microparticales functionalized by removing the core by adjust the PH at 2 by adding HCL 0.1M (overnight) Figure (4) after adding HCL microparticales shrinkage, after that adjust the PH at 4 for filling with Bromo stain then adjust pH at 8 to close the holes in the microparticales. The solutions of Urease enzyme were mixed with suspension of functionalized micro particles [12-13] and incubated for up to 1 hour. Figure (2) the micro-particles were mixed with florescence dye to have seen under florescence microscopy as shown in Figure 3.

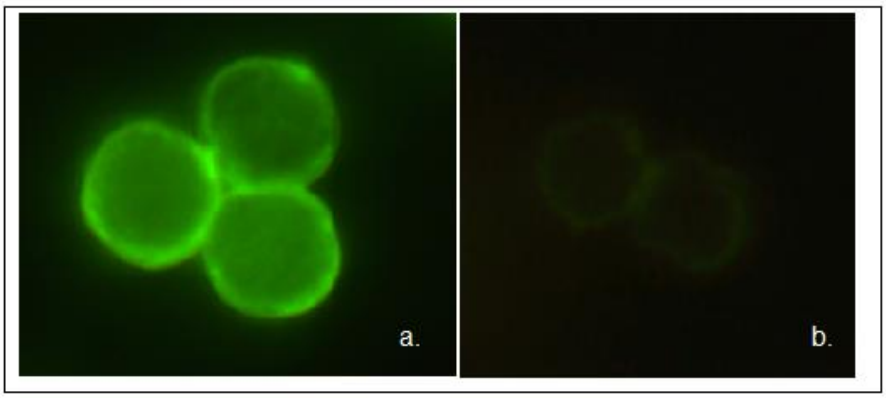

Figure 3: a. Capsules 6 u before add HCL. 100X. b. Capsules 6 u after add 0.1 M HCL (Lens magnification 100X). under the Fluorescence microscope.

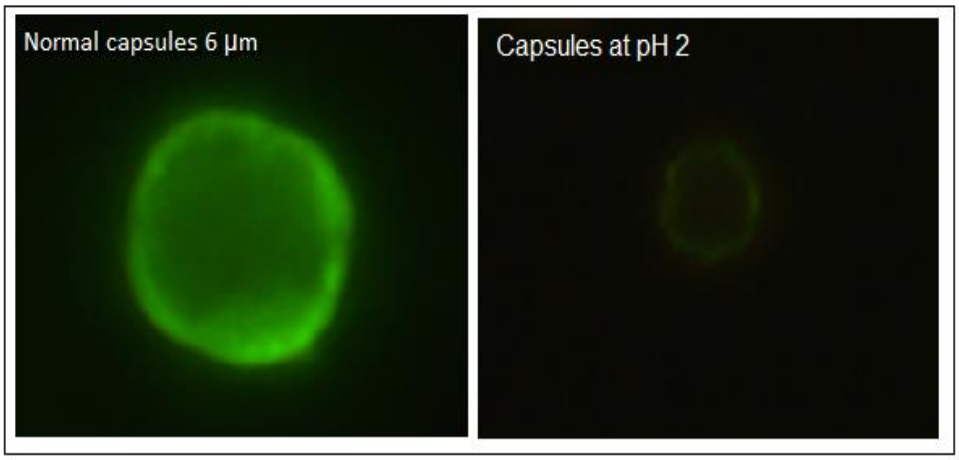

Figure 4: Capsules after & before add 0.1 M HCL

The Bromo dye is sensitive to the changes in pH its change from yellow to blue when Urease enzyme reactions with urea the solution change to alkaline then the dye change to blue (Figure 5). This high quality and fast detecting sensor.

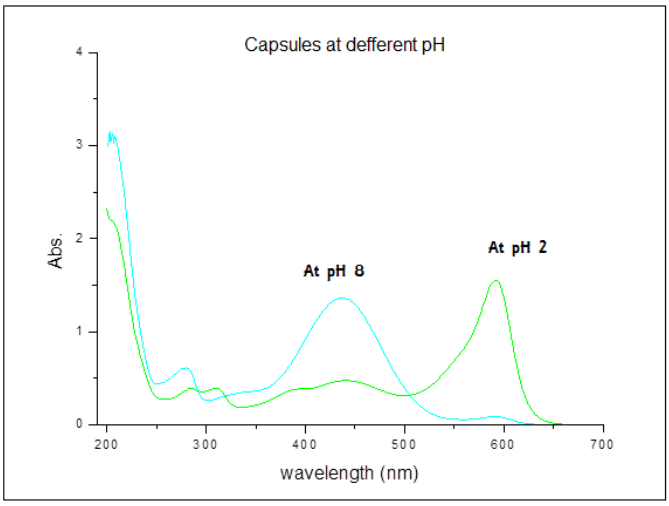

Figure 5: Micro particles with Bromo stain at different pH. Under the UV-vis.

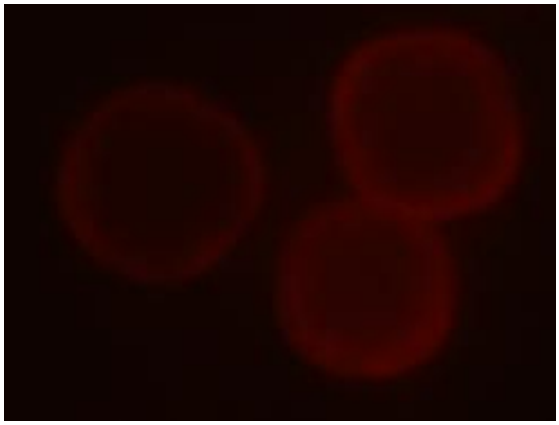

Figure 6: Microparticales under Fluorescence microscopy.

4. Conclusions

This work is succeed in developing the multilayer electrolyte on $MgCO_3$ through LbL Technology and then removed there cores by change in pH of the solution then filling the core with Bromo dye. In another case Bromo dye and the urease enzyme adsorbed on the surface of the microparticales. This procedure is promising to fabricate biosensors for many types of materials like heavy metals, Urease enzyme as a biosensor is a great attention because of its high sensitivity and stability, low price and short response time. The detection of Urea by Urease enzyme is based on the hydrolytic catalysis of urea by urease enzyme.

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أستخدام انزيم اليوريز لاكتشاف نسبة التلوث في المياه

رقية سليم العمار

المختبر المركزي – مديرية ماء البصرة

المستخلص

اليوريا لها دور مهم في استقلاب المركبات المحتوية على النيتروجين بواسطة الحيوانات وهو أهم مادة تحتوي على النيتروجين في بول الثدييات تم تحضير جهاز استشعار جديد لليوريا عن طريق ترسيب إنزيم اليوريز وصبغة برومو على سطح الجسيمات الدقيقة تم تحضير هذه الجسيمات الدقيقة من خلال ترسيب الطبقات الواحدة فوق الاخرى على التوالي بواسطة عملية التجميع الذاتي طبقة فوق اخرى .نجحنا في تطوير متحسس بايولوجي متعدد الطبقات متعدد الإلكتروليت يرسب مباشرة على MgCO3من خلال تقنية LbL وإزالة النوى هناك عن طريق تغيير الرقم الهيدروجيني للمحلول ثم ملئ اللب بصبغة برومو . في حالة أخرى ، تمتص صبغة برومو وإنزيم اليورياز على سطح الجسيمات الدقيقة.يعد هذا الإجراء لتصنيع أجهزة الاستشعار الحيوية لأنواع عديدة من المواد مثل المعادن الثقيلة ، وأجهزة الاستشعار الحيوية القائمة على اليوريز لها أهمية كبيرة نظرًا لحساسيتها العالية واستقرارها وسعرها المنخفض وقصر وقت الاستجابة.