

ENCAPSULATION OF UREASE IN MOLYBDENUM TRIOXIDE SOL-GEL

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ABSTRACT

Molybdenum trioxide sol-gel matrices were tested for their ability to host biomaterials in their structures without adversely affecting the activity of the encapsulated biomolecules. The sol-gel composites were prepared at room temperature; the urease was added during the hydrolysis step to ensure proper encapsulation in the pore structure of the sol. Phosphate buffer solution was added during this stage to maintain the pH value of the sol in the bio-compatible range. The activity of incorporated urease was tested by exposing composite sol-gel material to standard urea test solutions and measuring the amount of gaseous ammonia (released by the reaction of urea with water in the presence of urease) using an ammonia electrode. The concentration of urea solutions ranged from 1mM to 10mM. An electronic olfactory system (EOS 835) was also employed to detect the presence of gaseous ammonia. Gas sensing tests were done to verify the sensitivity of the matrix to ammonia. Microstructural analysis was carried out by means of scanning and transmission electron microscopy. It was found that urease retained its activity inside molybdenum trioxide sol-gels that are sensitive to ammonia. These hybrid nanoporous composites are useful in the field of biosensors and fuel cells.

INTRODUCTION

Most of the urea biosensors are based on the enzymatic action of urease (equation 1) which specifically catalyzes hydrolysis of urea and the product/s of the reaction are detected by various techniques [1-6].



The potentiometric sensor [7], which is most widely used, suffers from many drawbacks like slow response to change in NH_4^+ ion concentration and interference from other ions present in the solution like Na^+ , K^+ [8]. In some sensors, urease is immobilized in passive media (such as natural and synthetic polymers and passive sol-gel matrices) and the change in electrical conductivity or potential is measured using a probe electrodes.

The silica sol-gel method has emerged as a popular method of encapsulating bio-molecules as it is done at room temperature which is conducive for the bio-molecules [9]. The optical transparency of silica has been utilized for optical detection of pathogens [10-11]. Our approach is to utilize the gas sensitivity of the matrix to fabricate a resistive type bio-sensor, and this has not been tried before to the best of our knowledge.

Molybdenum trioxide was chosen as the host for bio-molecules as it has been shown in our earlier works to be sensitive and selective towards ammonia [12]. Here we report successful incorporation of urease in MoO_3 sol-gel matrix and sensitivity of bio-composite to ammonia.

EXPERIMENTAL

Urease solution was made by mixing 0.662g (10592 units) of urease [(EC 3.5.1.5) from SIGMA] in 25ml water + 25 ml glycerol. This procedure was based on reference [13]. Then for encapsulating the urease in sol-gel, 0.39104g of molybdenum isopropoxide [purchased from CHEMAT TECHNOLOGY INC] was added to solution containing 7 ml butanol [Butanol reagent ACS 99.4%(GC) from ACROS], 2 ml of 0.1M Phosphate Buffer Saline {PBS} buffer [PBS saline P-3183 from SIGMA] and 1 ml of urease solution. The mixture was placed in the ultrasonic cleaner for 2 hours and the sol was allowed to settle for 2 days in the refrigerator.

In order to assess the retention of the activity of urease in the sol-gel by potentiometric method, standard urea test solutions were prepared. 1 ml of sol was mixed with 20 ml of urea solution and 1 ml of ammonia pH adjusting Ionic Strength Adjuster (ISA), and then it was stirred by magnetic stirrer for 15 minutes. Five minutes later the voltage readings were taken using Thermo Orion ammonia electrode. The effect of each of the constituents on the potential was also studied. It was observed that while PBS lowered the potential difference, the pH adjusting ISA increased it, so all the readings were taken following the addition of ISA.

To detect the presence of gaseous ammonia released by hydrolysis of urea by urease, an electronic olfactory system (E-nose EOS 835- from SACMI) was used. The samples for this experiment were prepared by drying 1 ml of Sol-Gel in glass vials. Drying was done at room temperature for 2 days. 5 ml of 0.1M urea solution was then added to it and vials were covered with parafilm and were left for 2 minutes before analyzing the atmosphere using the E-nose. Comparisons were made between the response of sol-gel with urease encapsulated and sol-gel without urease.

In order to verify that the addition of urease did not affect the structure of the MoO₃, the ammonia sensitivity of the matrix of the bio-composite was tested. Gas sensing experiments were carried out at 300^oC. The samples for these sensing experiments were prepared by spin coating the bio-composite films on alumina substrate with gold electrodes. They were heat treated at 500^oC for 8 hours (this treatment denatured the enzyme as expected but was used to stabilize the orthorhombic phase of MoO₃). The gases used were Ammonia and nitrogen dioxide, the concentration ranged from 100 to 500 ppm. For XRD analysis, the same heat treatment was employed on the bio-composite material deposited on glass substrates.

TEM (Philips CM12) and SEM (LEO 1550) analysis were done for both heat treated and as received sol-gel composite.

RESULT AND DISCUSSION

Urease retained its reactivity (to catalyze the hydrolysis of urea) inside the molybdenum trioxide sol-gel. Figure 1 shows the concentration of urea versus activity plot which shows steady increase in activity with increase in urea concentration. The urea test solutions had concentrations in the range of 1-3mM so as to be able to detect urea levels that are below the “critical value” 50 mmol/L [14] of urea in blood.

In order to ascertain that the change in activity measured was due to catalysis of urea and not due to effects of other interfering ions present in PBS and pH adjusting ISA individual effects of both were studied and all the readings were taken after adding ISA.

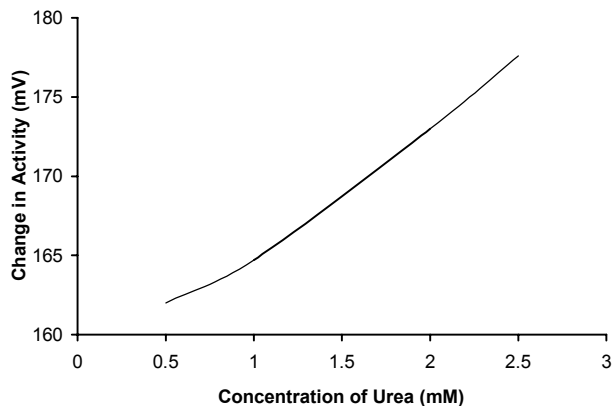


Figure 1. Activity of urease versus concentration of urea

The release of gaseous ammonia and reactivity of urease was confirmed by E-nose, comparison was done between atmospheres of sol-gel with Urease and sol-gel without urease. The composite caused higher drop in resistance figure 2. The reasons for the drop in resistance due to sol-gel without urease can be attributed to the release of entrapped alcohols on addition of water from urea solution or increase in the relative humidity. This is under investigation.

The gas sensing tests were done for the inorganic sol-gel matrix and it was found out that the bio-composite showed sensitivity towards ammonia. The behavior was n-type, which is typical characteristic of molybdenum trioxide.

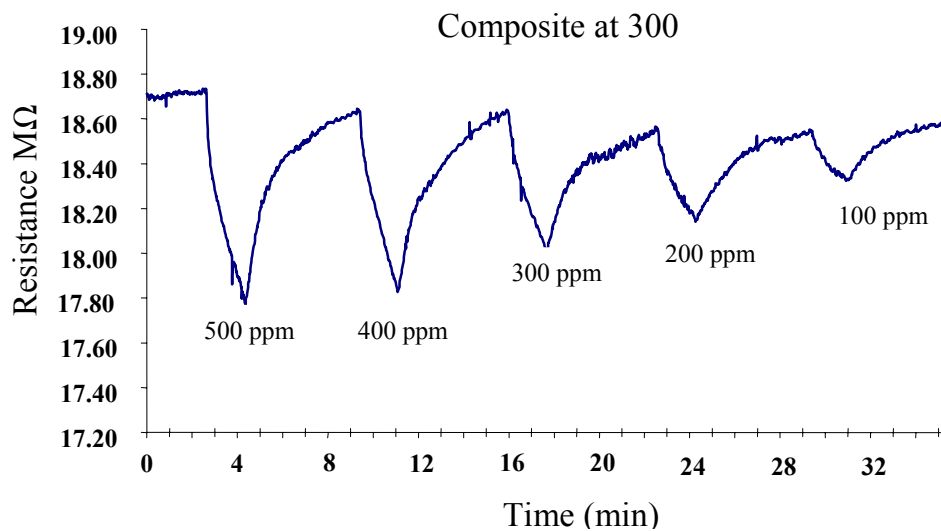


Figure 2. E-nose response shows drop in resistance in the sensor on exposure to atmosphere of sol-gel and urea solution

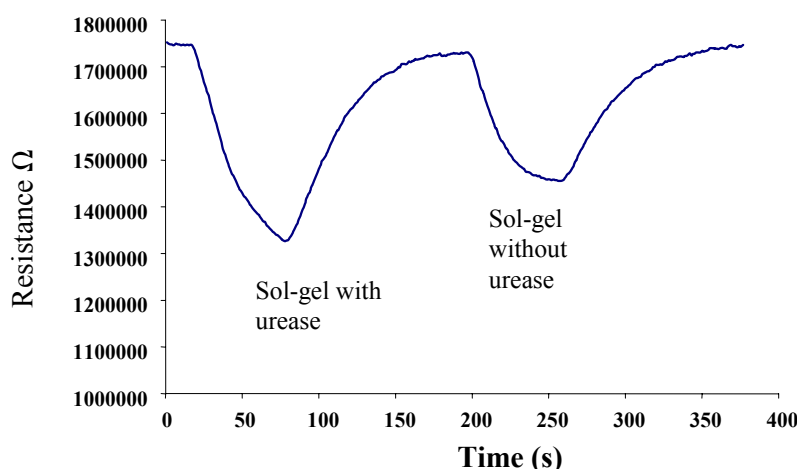


Figure 3. Gas Sensing test of composite sol-gel shows resistance as a function of gas concentration

Figure 4 shows the morphology of the sol-gel film with urease encapsulated within it. The image shows the surface structure of the sol-gel film containing biomolecules. Figure 5 shows the TEM image taken at higher magnification (125kX) showing clusters of molybdenum oxide formed upon drying of the sol-gel. These aggregates are porous as observed from the image. The size of the individual particles in each cluster is around 10-20 nm.

These porous nanocomposites are promising biosensing elements due to their unique properties, such as the functionality of the receptor biomolecule and the transducing active matrix.

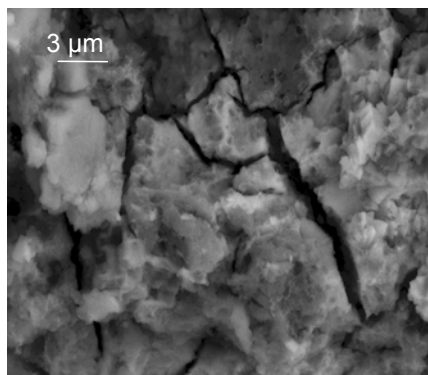


Figure 4 SEM image of sol-gel with urease Encapsulated

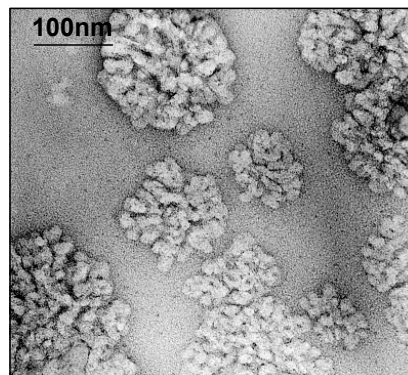


Figure 5 TEM image of dried sol-gel film with urease encapsulated

CONCLUSION

Urease was encapsulated in inorganic matrix of molybdenum trioxide, and it was found that urease retained its activity inside sol-gel. The matrix was found to be sensitive to ammonia. These bio-composite described here are useful for developing a resistive urea biosensor which is expected to be fast and selective unlike the potentiometric sensor. Furthermore, the resistive sensor response will be free from the presence of interfering ions in urea solution.

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