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NANOCOMPOSITE MATERIALS FOR ELECTRONIC NOSE

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ABSTRACT

Novel nanocomposite of enzyme in non-transparent metal oxide sol-gel matrix for resistive sensing was developed. Molybdenum trioxide sol-gel composites were prepared at room temperature; the urease was added during the hydrolysis step. 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 using a potentiometric ammonia electrode. The concentration of urea solutions ranged from 10mM to 1mM and the released ammonia caused a electrode potential to change by upto 20mV. These hybrid nanoporous composites are useful in the field of biosensors and fuel cells.

1. INTRODUCTION

Amount of urea in blood is an indicator of the functioning of kidney, elevated levels of urea in blood is indicator of many diseases. So a sensor which can rapidly and reproducibly measure urea is of considerable interest. There are many types of urea sensors currently available, the prominent among them being amperometric[1], conductimetric[2], FET based[3], potentiometric[4]. Though potentiometric sensors have become more popular than other due to general availability of instruments, they suffer from various drawbacks like they are slow to respond and ever slower to regain the baseline and are highly sensitive to the presence of any interfering ions like Na^+ or K^+ [5].

Our approach to solve the problem is to make a resistive sensor for urea. It is based on encapsulation of urease in the metal oxide matrix, so that on exposure to urea solution the urease encapsulated within the matrix will hydrolyze the urea into ammonia and carbon dioxide as shown below

$$NH_2CONH_2 + H_2O \xrightarrow{\text{Urease}} CO_2 + NH_3$$

The ammonia released can be detected by the metal oxide. Sol-gel processed molybdenum trioxide was chosen as the matrix as orthorhombic molybdenum has been shown to be highly selective ammonia sensing element[6].

2. 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 [12]. 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 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, standard urea test solutions were prepared. Thus 1 ml of sol was mixed with 20 ml of urea solution and 1 ml of ammonia pH adjusting ISA, and then it was stirred by magnetic stirrer for 15 minutes. Five minutes later the volt-age readings were taken using Thermo Orion ammonia electrode

To ensure that the activity detected was specifically due to the enzyme encapsulated and not because of the presence of other solution interfering chemicals, the individual effect of each component was considered. PBS caused the electrode potential to increase while pH adjusting ISA decreased the value of the electrode potential. Therefore, for all the readings the measured value of the potential after adding ISA was recorded. The value of the enzyme activity was calculated as the change in potential after adding sol-gel compared to the value of potential after adding ISA.

3. RESULTS AND DISCUSSION

Figure 1 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. Fig-ure 2 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.

Urease retained its reactivity (to catalyze the hydrolysis of urea) inside the molybdenum trioxide sol-gel. Figure 3 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 1mM so as to be able to detect urea levels that are below the "critical value" of urea in blood [7].

4. CONCLUSIONS

Bio-doped MoO_3 gels were prepared. It was observed that the enzyme retains its reactivity inside the sol-gel. The success of this step was vital for the synthesis of a resistive biosensor. The presence of the matrix consisting of the molybdenum oxide phase around the urease molecules is expected to greatly reduce the diffusion time of ammonia liberated from the hydrolysis of urea, to the metal oxide. The ammonia liberated can be detected in-situ, thus giving very fast response times

5. REFERENCES

[1] S. Sampath and O Lev, Anal. Chem, vol 68, pp.2015-2021, 1996

[2] N.F. Sheppard, Jr, D.J. Mears and A Guiseppi-Elie, Biosensors and Bioelectronics, vol 11, Iss 10, pp. 967-979, 1996

[3] L.D. Watson, P. Maynard, D.C. Cullen, R.S. Sethi, J. Brettle and C.R. Lowe, Biosensors, vol 3, pp. 101-115, 1987/88

[4] J.M.C.S. Magalhães, A.A.S.C. Machado, Talanta, vol 47, pp.183-191, 1998

[5] G G. Guilbault, M.L. Seo, Talanta, vol 41, Iss 6, pp.1029-1033, 1994

[6] A.K. Prasad , P.I. Gouma, Journal of Materials Science, vol 38, pp. 4347-4352, 2003

[7] W.J. Johnson, W.W. Hagge, R.D. Wagoner, R.P. Dinapoli, J.W. Rosevear, Mayo Clin Proc, vol 47 Iss 1, pp. 21-29, January 1972

FIGURES

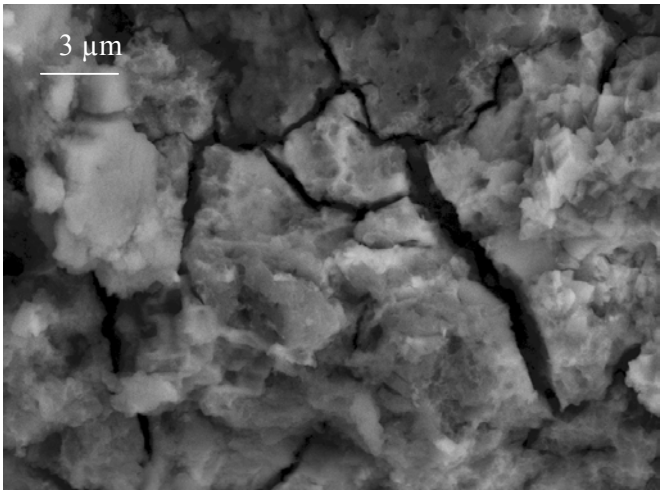

Figure 1. SEM image of sol-gel film with urease encapsulation

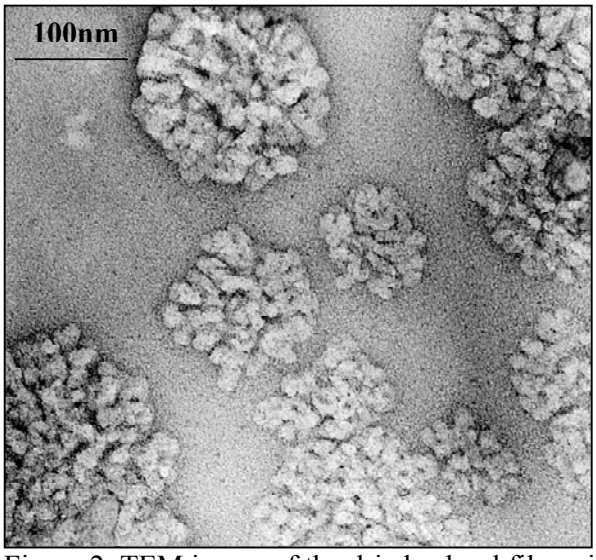

Figure 2. TEM image of the dried sol-gel film with urease encapsulated inside the aggregates

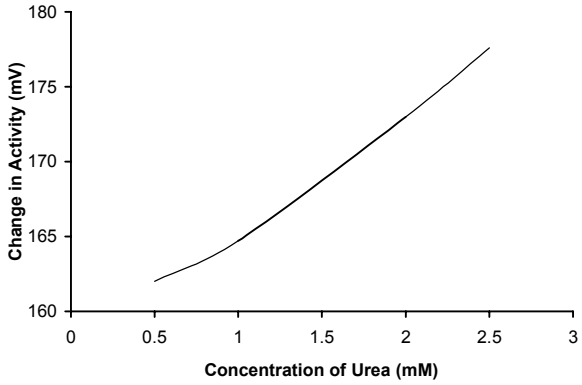

Figure 3. Activity of Urease in sol-gel versus urea Concentration