Highly Porous Sol-gel Based Urea Biosensor

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Abstract **– Here, we are reporting a highly porous sol-gel bioceramic based disposable urea biosensor where urease enzyme is entrapped into the honeycomb sort of porous structure of silica sol-gel. To hinder denaturation of enzyme, a biopolymer was supplemented at low-temperature into sol-gel layer, moreover, which raised its porosity in contrast to catalyst based sol-gel and skipped over irresistible characteristics of this ceramic silica, crispness and striction. Structural variations of different sol-gel layers were confirmed by scanning electron microscopy. For the impedimetric study, gold electrodes were structured onto the floor of alumina by screen-printing technique. The behaviour of the biosensor for urea was studied by electrochemical impedimetric method. In contradiction to base catalyst based sol-gel, this polymer based silica sol-gel gives much more improved results. In contrast to conventional methods, this biosensor required 1 drop of urea sample for detection. It had small surface area of 1cm^2, very low response time of 2-3 seconds and lower detection limit of 0.001mM.**

Keywords – urea, sol-gel, porous, bioceramic, biosensor, screen printing, impedimetric

I. INTRODUCTION

Wavering of urea level in clinical chemistry, environment, milk, cosmetics, agriculture as well as food lead to various troublesome situations where human can not run from [1, 2]. Hence, there is an immense requirement of a proficient urea biosensor.

In this research paper, equation (1) is utilized as the fundamental reaction on which whole sensor relies. The principle lies behind the hydrolysis of urea in the presence of urease enzyme which gives rise to various ions.

$$NH_2CONH_2 + 3H_2O \dashrightarrow 2NH_4^+ + HCO_3^- + OH^- \quad (1)$$

Various immobilization techniques for urease encapsulation like adsorption, covalent bonding and cross-linking and sol-gel encapsulation are reported [3-5]. Holding the facts in mind that care for chemical structure of biomolecules is inessential, requires no consumption of unsafe chemicals that can denature the immobilized biomolecule deficiently, exclusive feature of modifiable pore size depending upon the dimension of biomolecule and minor response subtraction due to discharge of the enzyme from the solid support, silica sol-gel encapsulation technique is exceptionally better than rest of the methods [5-10].

II. METHODOLOGY

A. Materials

Urease (EC 3.5.1.5 from jack beans) and urea were purchased from SRL. Remaining chemicals were of analytical grade and purchased from Merck.

B. Development of Urea biosensor

Construction of gold interdigitated electrodes were accomplished (Fig. 1) using screen printing technique on an alumina substrate. Then, electrodes were cured at 500 ^{o}C for one hour.

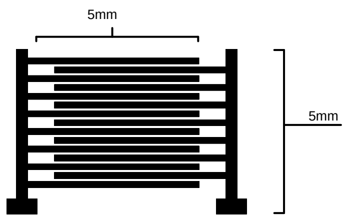

Fig. 1 : Interdigitated electrodes

(1) Base catalyst silica sol-gel solution

2ml Tetraethylorthosilicate (TEOS), 1ml ethanol, 1ml distilled water and 0.05 ml ammonia were magnetically stirred in a beaker for one more hour at room temperature.

(2) Polymer based silica sol-gel

1ml polyethylene glycol (PEG), 2ml Tetraethylorthosilicate (TEOS) and 2ml ethanol were magnetically stirred in a beaker for one more hour at room temperature.

Meanwhile, in both solutions, phosphate buffer was added to maintain pH at 7. 100ul urease enzyme was intermingled with 100ul prepared sol-gel solution. Further, resulting solution was applied onto the working area of the biosensor. The biosensor was stored at 4 ^{o}C for 1 day. Base catalyst and polymer sol-gel based biosensor is named, here, base biosensor and polymer biosensor. The biosensor's impedimetric response was investigated by Keithley 4200-SCS semiconductor system in frequency range 1KHz-1MHz at 5mV.

III. RESULTS

A. Characterization Studies

Fig. 2 is the SEM image of base catalyst silica sol-gel where, due to ceramic nature, rough surface with less number of pores is obtained. Accumulation of some colloidal matter can be seen in the image. Rather, in contrast, Fig. 3 show the presence of a uniform pattern of spherical pore per unit area with an average diameter of around 1µm in PEG based silica sol-gel.

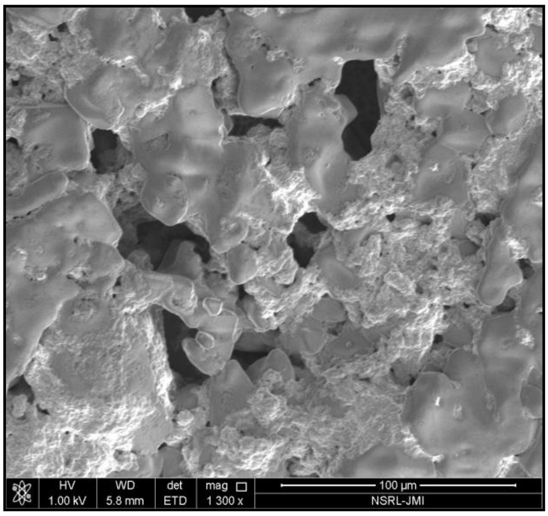

Fig. 2 : SEM image of base catalyst based silica sol-gel

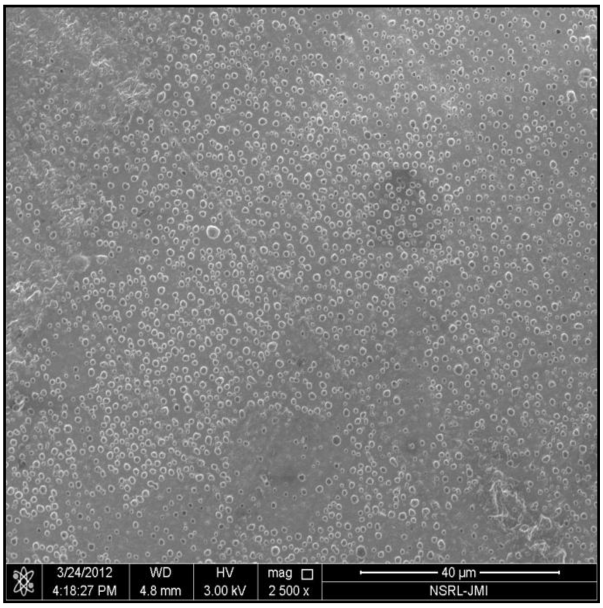

Fig. 3 : SEM image of PEG polymer based silica sol-gel

B. Impedimetric Studies

After the impedimetric examination, we found that the entrapment of urease enzyme into polymer based sol-gel layer reduced the impedance of the biosensor as shown in Fig. 4. When electrically charged enzyme is added into sol-gel layer of biosensor, the overall capacitance of the system raises as electrical permittivity of layer due to enzymes increases. Thereby, impedance, which is inversely proportional to capacitance, reduces. Furthermore, these enzymes increase conductivity that decrease resistance as well as impedance. Hence, gross impedance decreases. Since, for impedance, contribution of capacitance is frequency dependent. It is high for frequecies below 10KHz which is called capacitive region as compared to resistance parameter. For frequencies beyond 10KHz known as resistive region, the dominant parameter is resistance over capacitance.

In Fig. 5 & 6, Withal, exposure to urea solution, the impedance of the devices decreased further. This change in impedance is due to hydrolysis of urea and formation of ammonium ions in the presence of urease. Simultaneously, the figures show the comparison between base biosensor and polymer biosensor. As we can observe that there is much more drastic reduction in impedance of polymer biosensor in contrast to base biosensor. This may be due to its raised porosity after the addition of PEG, furthermore, which leads to increased capacity to retain urease enzyme into the pores.

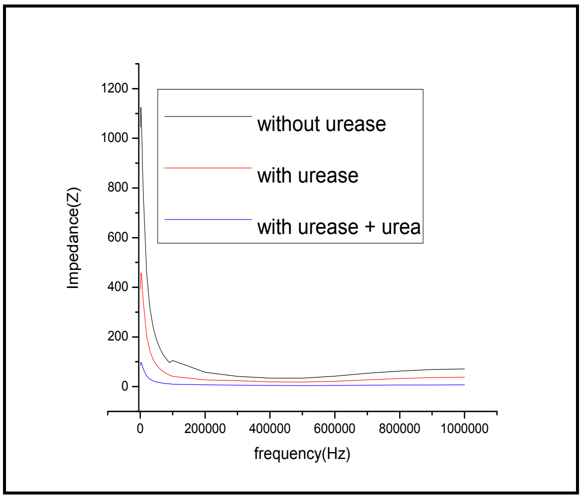

Fig. 4 : Impedance decrease with each step of development of biosensor in polymer based biosensor

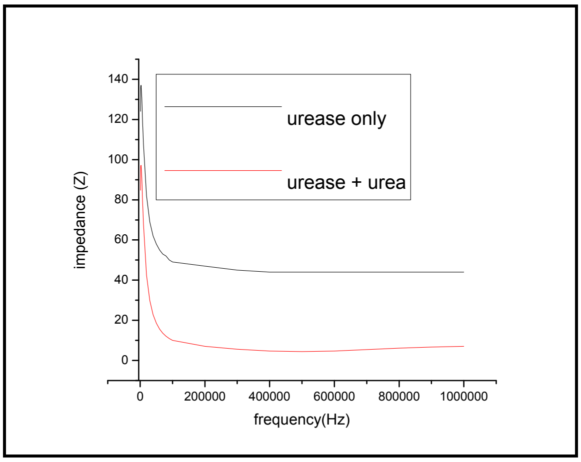

Fig. 5 : Impedance decrease with increase in urea concentration in polymer based biosensor

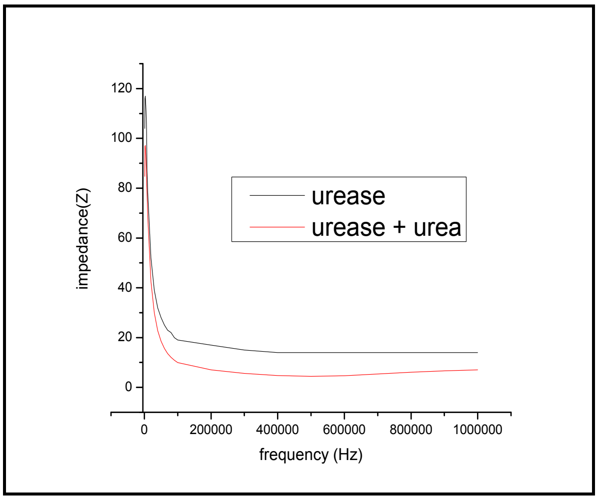

Fig. 6 : Impedance decrease with increase in urea concentration in base catalyst based biosensor

The working of biosensor can be explained by an equivalent circuit of the impedance measurement system (Fig. 7). Remaining frequency dependent, at lower frequencies (<10KHz), capacitance offers more impedance in contrary to resistance and the current goes through low impedance path following R_{ct}, R_s and R_{ct}. At frequencies higher than 10KHz, the strike of resistive impedance is high in comparison to capacitive. Meanwhile, the capacitive impedance reduces with increase in frequency. So, the current flows through capacitance C_{di}.

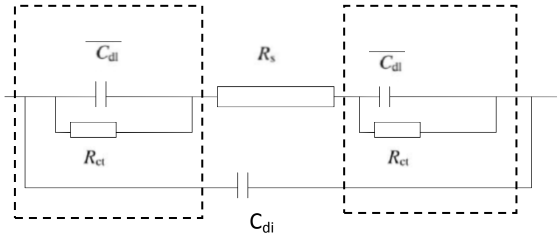

Fig. 7 : The equivalent circuit for the impedance spectroscopy measurement with the electrode. double layer capacitance (C_{dl}), charge transfer resistance (R_{ct}), resistance of the electrolyte solution (R_s) and dielectric capacitance (C_{di})

IV. CONCLUSION

From the research attempt, it can be successfully concluded that PEG based silica sol-gel not only omitted the relevant shortcomings of silica sol-gel, brittleness and shrinkage, moreover, improved the response of the biosensor. Supplementation of PEG in silica sol-gel solution amplified the porous network which further improved the capacity of enzyme entrapment. We have confirmed that the interdigitated electrodes could be used to check the impedance change. Impedimetric studies show that our highly porous sol-gel based urea biosensor has shown meaningful deviation in impedance in the presence of urea. Hence, this urea biosensor appears to be appropriate for advance testing in health practice because of its satisfactory outcome.

V. ACKNOWLEDGMENT

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VI. REFERENCES

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