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DEVELOPMENT OF NANO-MATERIALS FOR THE IMMOBILIZATION OF BIOMOLECULES

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ABSTRACT

Nanotechnology is a fast growing field with great promises for providing us many breakthrough that will change the direction of technological advances in a wide range of application. There are two general approaches for the synthesis of different nanoparticles and development of nanostructures. These approaches are: top-down or bottom-up strategies which open up novel avenues for myriads of applications. Bottom-up fabrication methods include the miniaturization of material components to the atomic scale with further self-assembly process leading to the construction of nanostructure. In the present work, the urea sensing film formed by encapsulated enzymes show potentiometric response of the modified gold electrode (Urs/Alginate/Nano-Au) that depends on the mass transportation of the substrate (urea) to the mod+ified workin−g electrode containing encapsulated urease and forming electro-active species

KEYWORDS: Nanotechnology, nanostructure, substrate, Bottom-up fabrication.

INTRODUCTION

Nanotechnology is a fast growing field with great promises for providing us many breakthrough that will change the direction of technological advances in a wide range of application. It is the foundation for the development of useful functional systems at the nanoscale level. These materials can have 0D, 1D, 2D or 3D (D•dimension) depending upon their general shapes and demonstrate distinct physical, chemical, electronic properties that are different from bulk material. These properties can be used to fabricate innovative and upgraded enhanced targeted therapy systems, gene delivery and molecular imaging, drug delivery, biomarker mapping, sensing devices precisely, electrochemical sensors and biosensors.

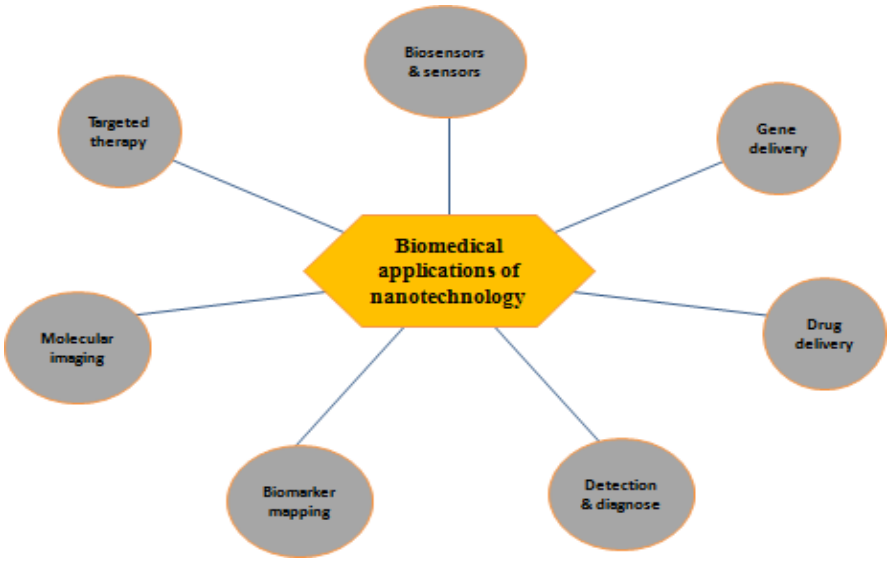


Figure 1: Overview of biomedical applications of nanotechnology.

A far reaching scope of nanomaterials, particularly nanoparticles with different properties have found wide application in different areas of diagnostic techniques and analytical methods. In these devices, the smart handling of nano-particle enhances the performances through improved sensitivity of measurement and lower detection limits of several orders of magnitudes. The advantageous property of nanoparticle is their high specific surface area, high loading efficiency, and ease of modification of particle surfaces which permits the immobilization of an increased amount of biomolecules.

Approaches for nanomaterial development

There are two general approaches for the synthesis of different nanoparticles and development of nanostructures. These approaches are: top-down or bottom-up strategies which open up novel avenues for myriads of applications. Bottom-up fabrication methods include the miniaturization of material components to the atomic scale with further self-assembly process leading to the construction of nanostructure. The forces at nanoscale combine to form self- assembled units which further grows into larger structures e.g. formation of nanoparticles from collod dispersions. For a major part of utilizations, these strategies have found optimal trade-offs between durability, mechanical properties and other functions such as density, permeability, colour, hydrophobicity, etc.

In top-down synthesis, the destructive methodology is utilized wherein larger (macroscopic) structures, which can be externally controlled, are processed into smaller nanostructures. Typical examples are etching through mask or ball milling. Here, the three types of destructive forces are involved in re-structuring macromolecules or large particles into smaller ones such as shear impact, pressure or compression which are used in industries to develop polymeric particles. But it shows many drawbacks such as high cost of equipment and hardware which needs expensive maintenance. Thus, it is a great challenge to develop cost effective strategies to synthesis nanoparticles with well characterized properties. Today, the potential of nanomaterials is reflected by the fact that many of them are entering a variety of markets. Thus, starting from molecular precursors or well-defined nano-building blocks, the materials are processed directly as particles, fibres, coatings, foams or monoliths. These can be obtained by applying micro-patterning technology or by developing hierarchial structures by coupling colloidal fluids with the help of physical chemistry of complex fluids, soft matter. An over- view of top-down strategies for nanomateial fabrication. Various top-down strategies can be used to develop nanoparticles for example, trypsin has been effectively encapsulated by shredding Ca-alginate beads to microparticles of diameter~200 μm with the help of food processor. By utilizing homogenization technique, an emulsion can be shaped by blending two immiscible liquids utilizing a mechanical device. Once the emulsion is formed, gelling is done either by temperature treatment or by utilizing gelling agents for example, Ca^{2+}, Mg^{2+}, Ba^{2+}, Cu^{2+}, Zn^{2+} and Al^{3+}, or fermentation operators, for example, glucono-delta-lactone. Monovalent cations can likewise be utilized to induce gelation upheld by electrostatic screening. In expulsion, a biopolymer arrangement is constrained into a gelling environment through a nozzle and can be scaled up to a modern scale to create biopolymeric particles.

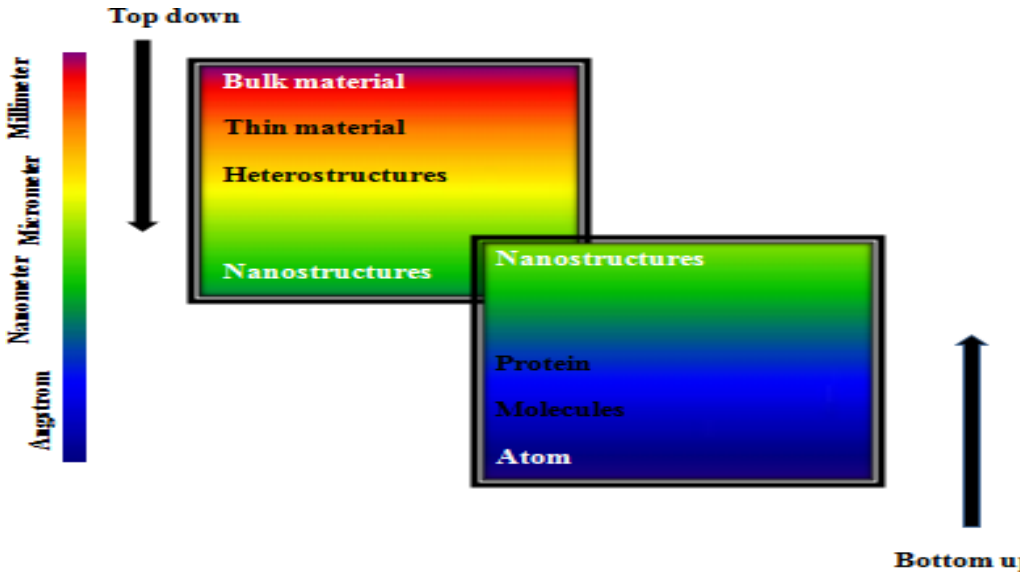


Figure 2: Directional nature of top down and bottom up synthesis for the development of nanostructures.

Though in a research laboratory condition, biopolymer arrangement is stacked up into a syringe and expelled through a needle into a solution accordingly advancing gelation can create biopolymeric particles. The particle size can be reduced by giving syringe and hydrocolloid arrangement an inverse charge or else polymeric arrangement can be separated by utilizing ultrasound. Gellan gum globules e.g. have as of late been produced utilizing the expulsion strategy.

Experimental Materials and methods

Urease, sodium alginate, and hexane purchased from sigma Aldrich. Polyoxyethylene sorbitan mono-oleate (Tween®80), $CaCl_2$, $SrCl_2$, $BaCl_2$, $MnCl_2$, $FeCl_2$, $CoCl_2$, $CuCl_2$, NaCl and urea were supplied by Fisher Scientific. Tris-acetate and acetic acid were obtained from Qualigens India. All chemicals were used as received. Double distilled water was used throughout the experiments.

Apparatus

Nano-gold elecrode (nano-gold thin film deposited on glass-slide from Optochem International, India), platinum wire, Ag/AgCl (3M KCl) was used as working electrode, counter electrode and reference electrode respectively. Laboratory measurements were performed in Bob`s electrochemical cell with a three-electrode configuration by using a Gamry Instruments ESA 410 (Germany) controlled by a PC computer via Gamry software.

Fabrication of enzyme electrode

Preparation of alginate nanogels using Ca^{+2}, Sr^{+2}, Ba^{+2} and Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+} as cross- linkers and encapsulation of urease were done as reported earlier.[26] The alginate nanogel/enzyme encapsulated alginate nanogels cross-linked with different divalent cations (Ca^{+2}, Sr^{+2}, Ba^{+2} and Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}) was suspended in Tris-acetate buffer saline solution by sonication for 15 minutes. The alginate nanogel was further drop casted on the surface of gold electrode to form a thin film on the square area of 0.5 mm length and was allowed to equilibrate at 4˚C for one hour and then dried at room temperature. Three successive coatings of the casting solution were done on the sensing surface of the gold electrode to obtain stable film. Enzyme coated gold electrode was stored in Tris-acetate buffer at pH 7.2 and at 4˚C when not in use. After each use enzyme coated electrode was rinsed with deionized water.

Surface characterization by SEM measurement

Scanning electron micrographs (SEM) were obtained with a ZEISS EVO 18 series scanning electron micrograph model EVO50 at an acceleration voltage of 20.0 kV.

Experimental set-up and potentiometric measurement

Potentiometric measurement was done on a Gamry potentiostat MODEL No. ESA 410, USA. The experiments were carried out in a conventional three-electrode cell configuration consisting of a working electrode (Urs/alginate nanogel/Au), Ag/AgCl reference electrode and a platinum wire as a counter electrode. In the electrochemical cell, enzyme coated electrode was dipped into 5.0 ml of Tris acetate buffer-saline (pH=7.2, 100mM) and corresponding outer circuit potential (OCP) was determined. Subsequently, urea solution of known concentration was added one by one from the concentration range 1mM to 30mM and corresponding change in OCP were further measured to obtain a calibration curve.

Measurement of optimum pH for urea biosensor

Effect of pH was studied in 100mM Tris acetate buffer saline between pH 6.0-9.0. In the electrochemical cell, potentiometric measurement was done as described above. By adding 1 mM urea solution to Tris acetate buffer-saline at different pH and 100mM concentration, corresponding change in the OCP was measured.

Effect of buffer concentration on urea biosensor

Effect of buffer concentration was studied in Tris acetate buffer saline by calibrating the enzyme coated electrode from 50mM to 500mM buffer solution at constant pH of 7.2. The change in potential was observed by adding 1-30mM urea solution to each enzyme coated electrode at different concentration of Tris acetate buffer saline and measuring the subsequent change in the OCP.

Measurement of effect of interferent's

Effect of interferent`s measurement was done by introducing various interfering species into the test solution containing 10mM urea at 100mM Tris acetate buffer saline at constant pH~7.2. Consequently, OCP response of the biosensor was studied and change in the potential was observed.

Measurement of urea in blood serum sample

Various key parameters important for the successful operation of biosensor was optimized (i.e. effect of pH on the OCP, suitable buffer concentration and effect of interferents on OCP) for urea measurement using fabricated biosensor. The performance of biosensor in clinical blood serum samples were studied by adding 100µl of serum samples to the buffer saline solution (pH~7.2, 100mM) instead of known urea solution and OCP was determined as described above. Serum was not diluted before potentiometric measurement. All measurements were repeated six times at 25˚C±1 and standard deviations of repeated data were calculated.

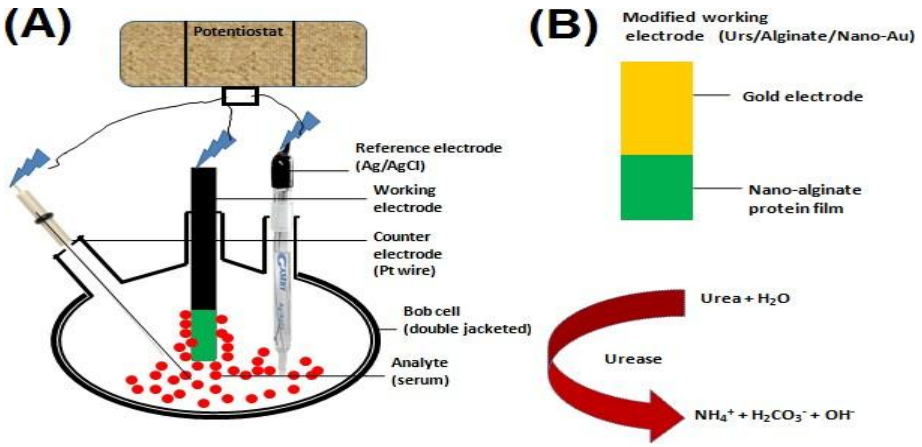
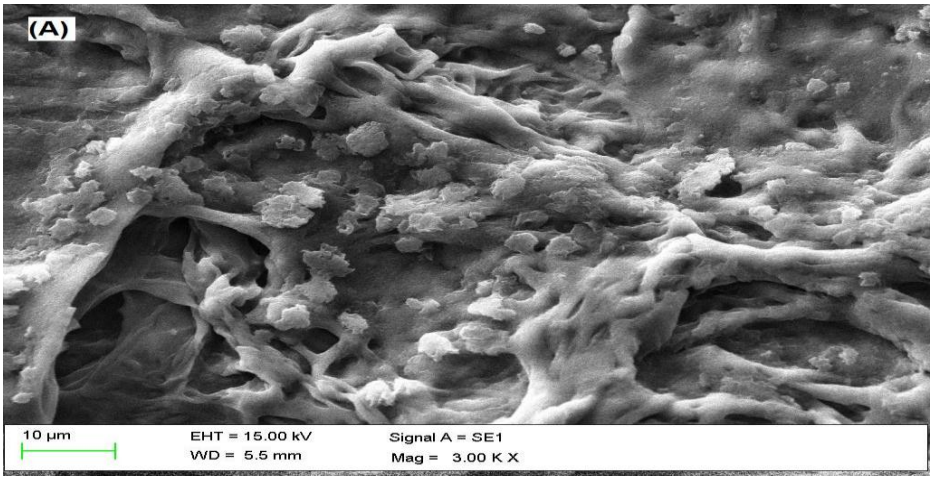


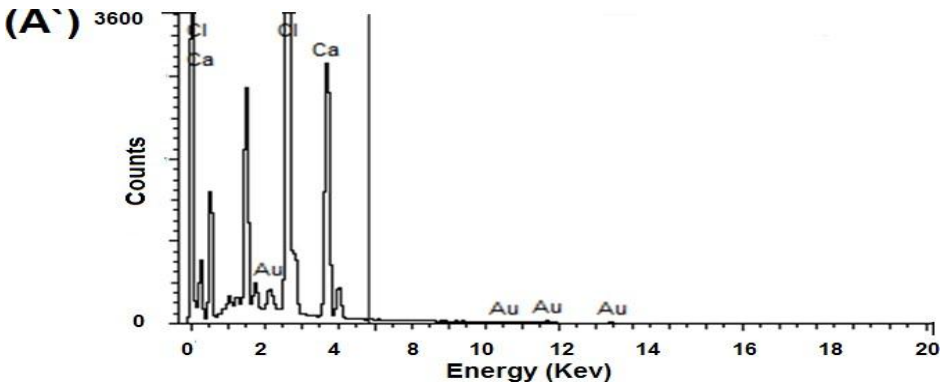
Figure A: Schematic diagram of experimental set-up used for urea determination. B: Modified enzyme electrode (working electrode) used as a biosensing element.

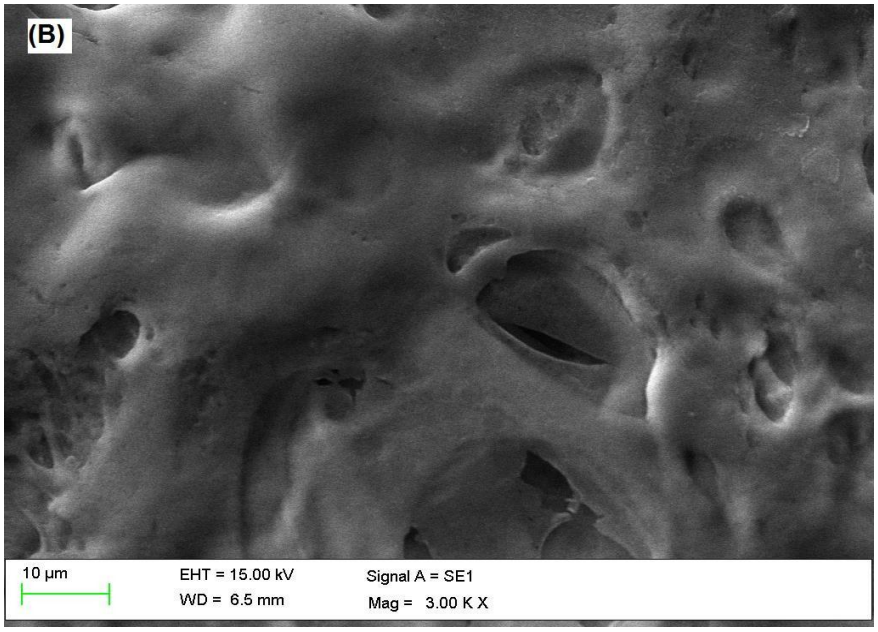
RESULT AND DISCUSSION

Characterization of enzyme electrode using SEM

The modified working electrode was developed by drop-casting the different alginate nanogel suspension on to the nano-gold surface. The surface feature of film, formed on the gold electrode was studied by scanning electron microscopy. SEM micrographs A-G. The fabricated working electrode showed porous, dense structure at the surface formed by drop-casting of Ca-alginate consisting of scattered small spherical grains like structure. Ba-alginate nanogels based modified electrode is more granular and porous at the surface while the modified electrode surface formed by Fe-alginate, Co-alginate and Cu-alginate nanogels is flexible and scattered. The surfaces of Sr-alginate and Mn-alginate nanogel based films are almost smooth, homogeneous and without pores in appearance. The porous, dense films are advantageous for electrochemical measurement in heterogeneous systems as it might supply larger surface area for enzyme–substrate catalytic reaction which affects response signal. The corresponding EDX spectra A`-G` below the SEM micrographs show the presence of corresponding divalent cation which acts as a crosslink in the drop-casted film showing the polyelectrolyte nature of the film.



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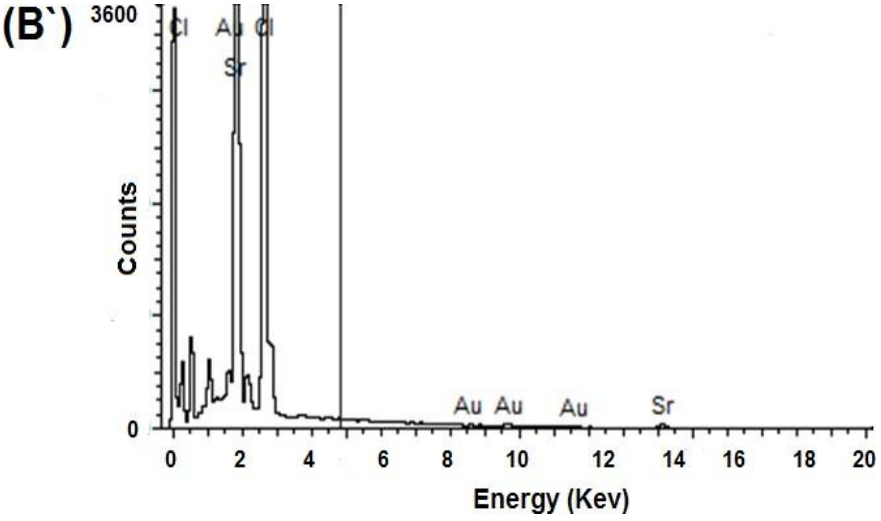
WD = 6.5 mm
Signal A = SE1
Mag = 3.00 K X


(B`)
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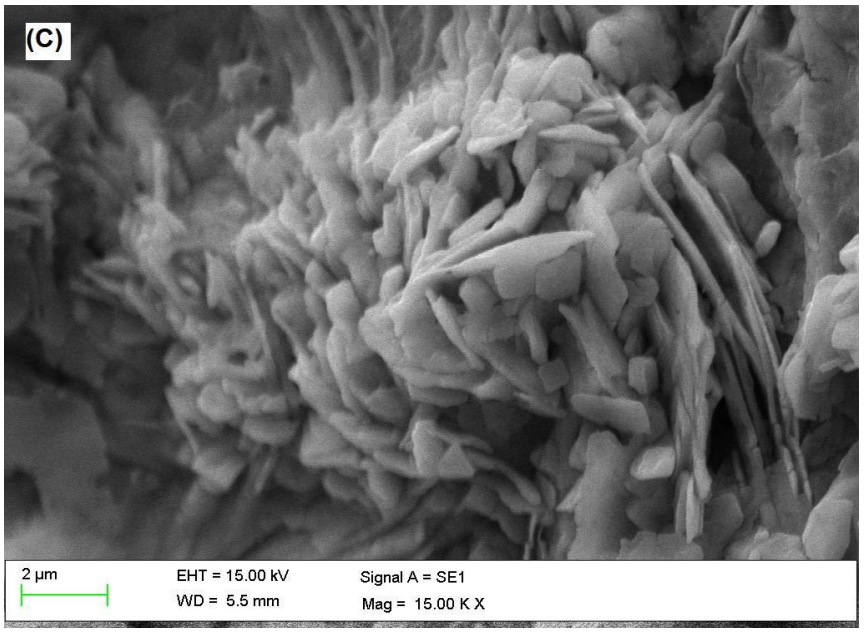
Cl
Au
Cl
Sr
Counts
0
Au Au Au Sr
0 2 4 6 8 10 12 14 16 18 20
Energy (Kev)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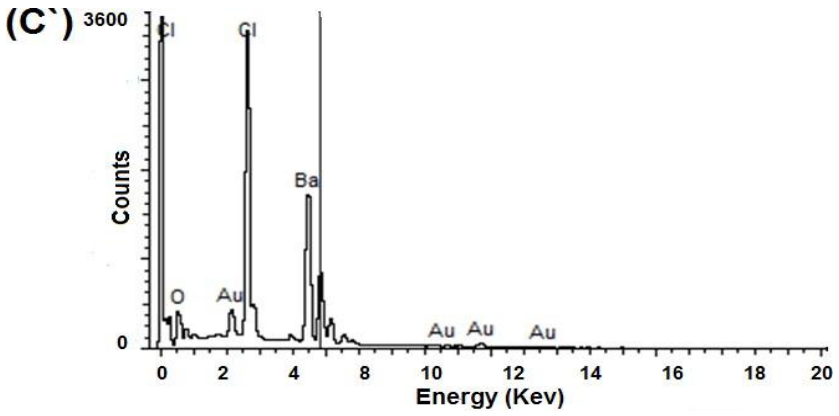
(C`)
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Ba
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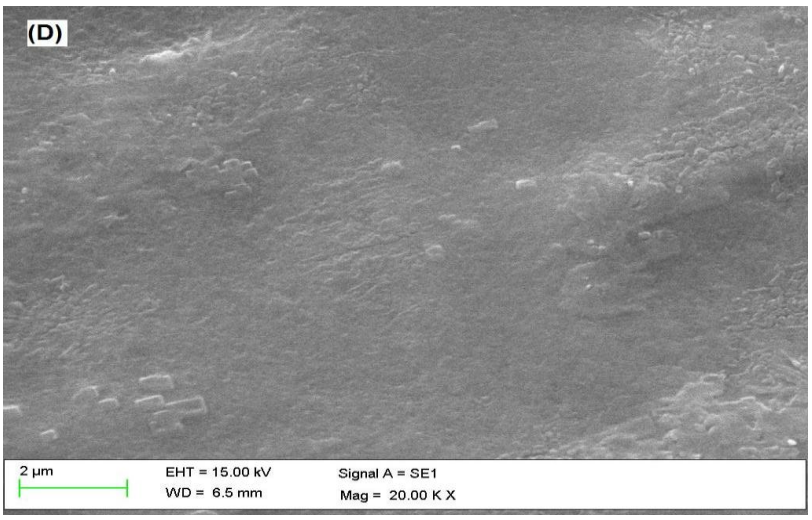
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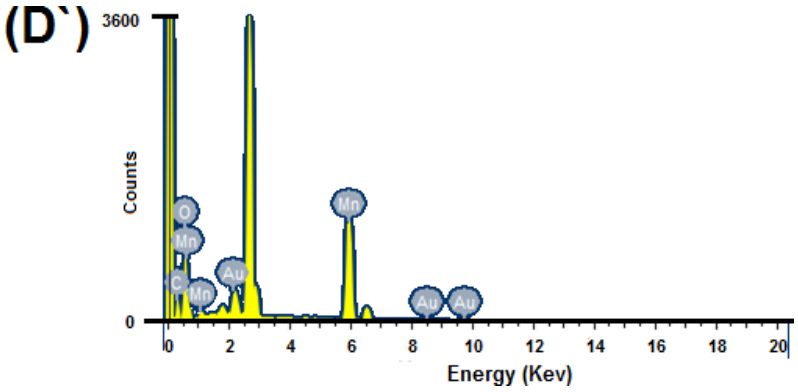
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Counts
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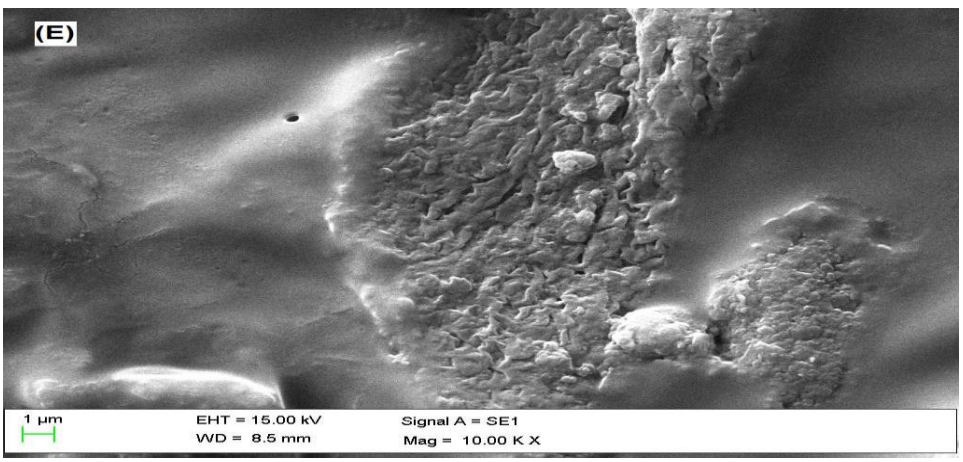
Au
Mn
Au Au
0
0 2 4 6 8 10 12 14 16 18 20
Energy (Kev)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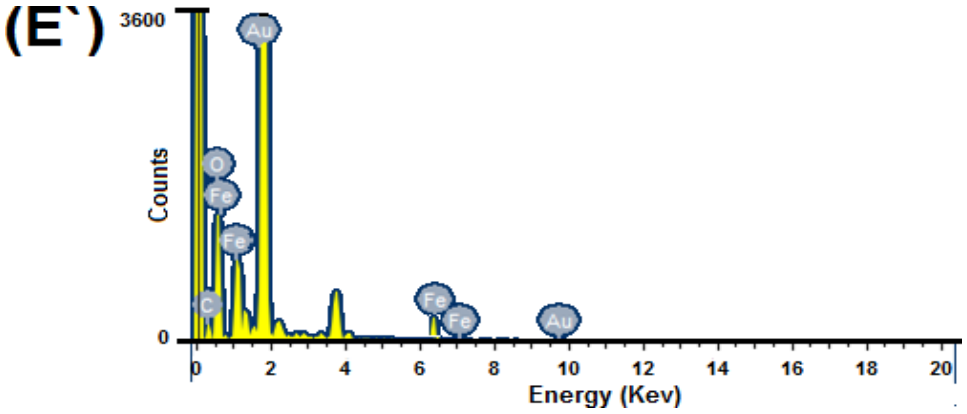
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Counts
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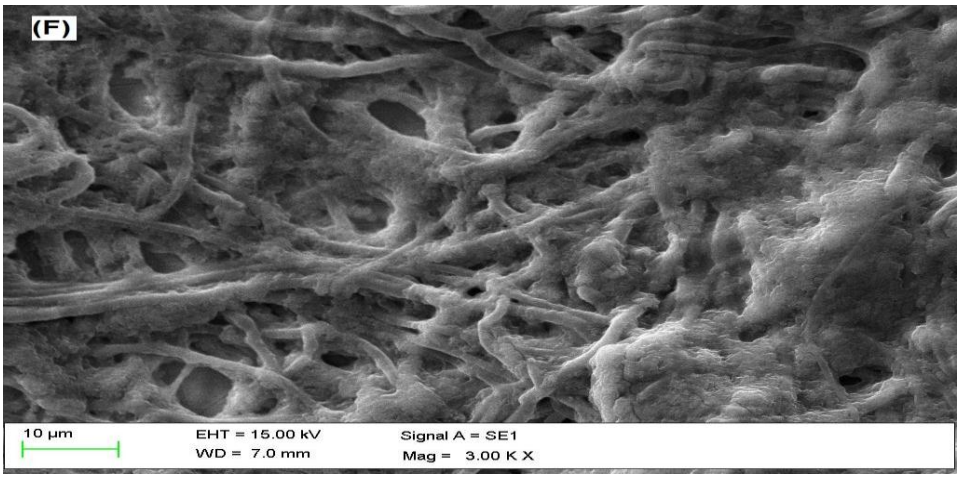
Au
O
Fe
Fe
C
Fe
Fe
Au
0 2 4 6 8 10 12 14 16 18 20
Energy (Kev)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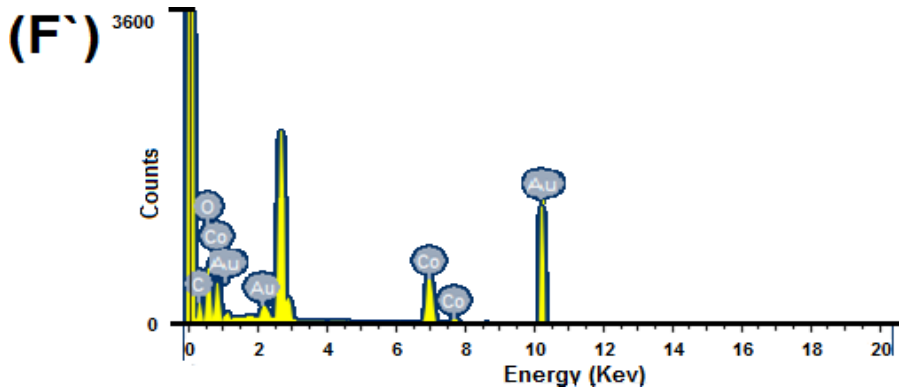
Mag = 3.00 K X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Counts
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Au
C
Au
Co
Co
Au
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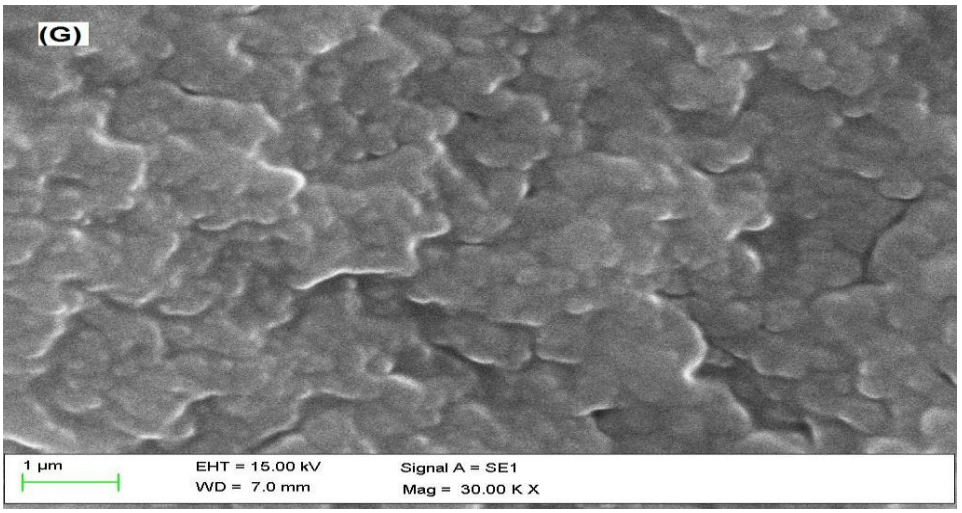
Energy (Kev)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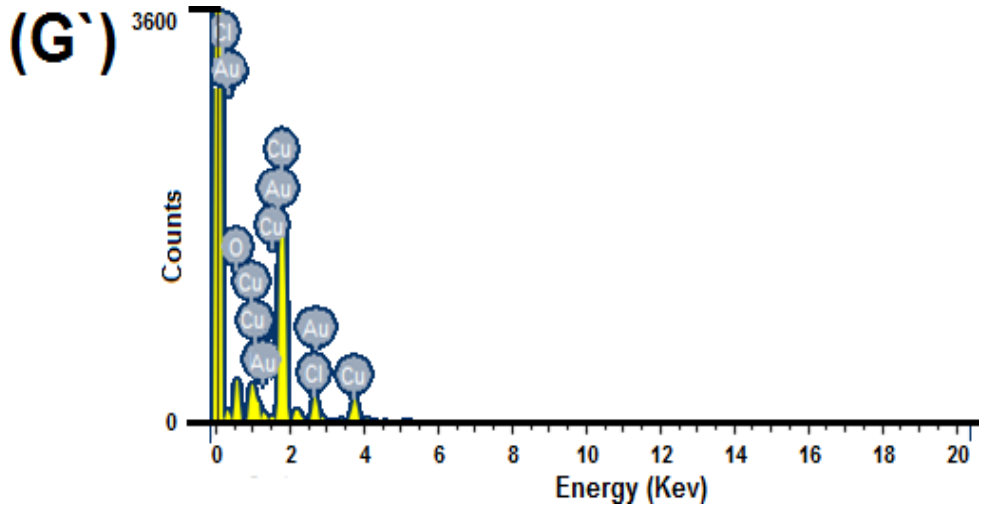


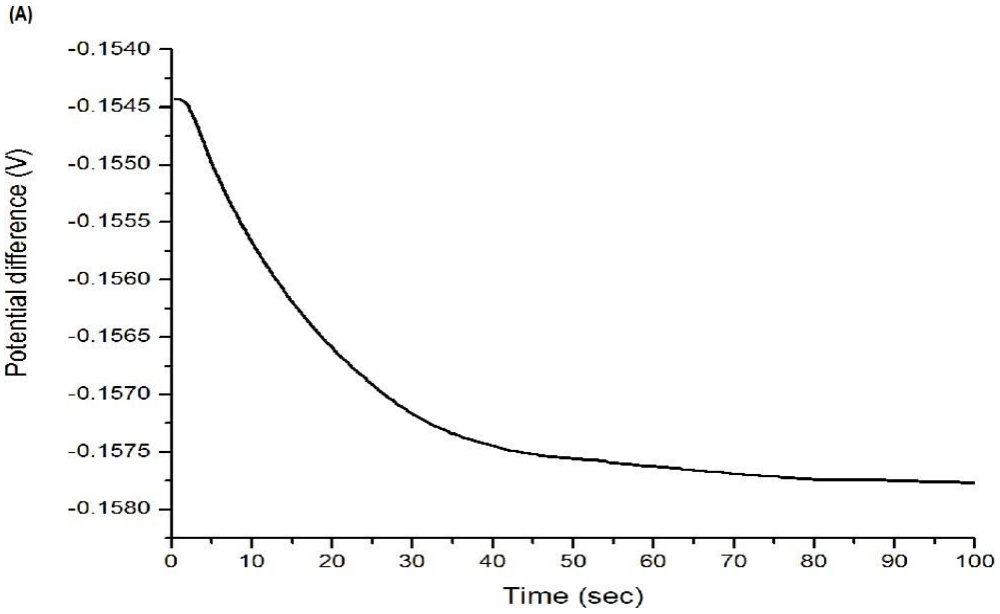
Figure: SEM micrographs and corresponding EDX spectra (A′-G′) of modified electrode (Urs/Alginate/Au-Nano) containing film of A. Ca-alginate, B. Sr-alginate, C. Ba-alginate, D. Mn-alginate, E. Fe-alginate, F. Co-alginate, G. Cu-alginate.

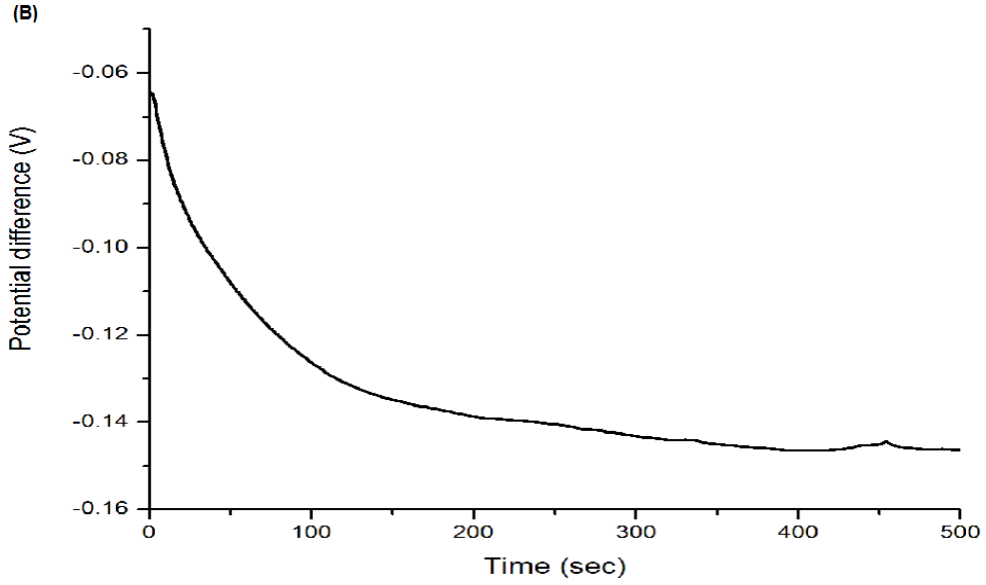
Potentiometric measurement and electrode response characteristics

The potentiometric response of urea biosensor is based on the biochemical reaction of urea hydrolysis catalyzed by Urease (Urs). These measurements were done in an electrochemical cell with three electrode system where Ag/AgCl was used as reference electrode, platinum wire as counter electrode and alginate film coated electrode (Urs/Alginate/Au-Nano) as working electrode. The urea concentrations were determined by measuring the outer circuit potential (OCP) changes observed due to ions produced in the enzymatic reaction. Similar studies are reported by Farghaly, A. A., Lam, M., Freeman, C. J., Uppalapati, B., Collinson, M. M.,[21] on the planar gold and nanoporous gold electrode using normal impedance voltammeter where OCP is used for the measurement of ascorbic acid concentration. Ahuja, T., Kumar, D., Singh, N., Biradar, A. M., Rajesh, reported the application of ITO electrode for the development of potentiometric urea biosensor by immobilizing urease enzyme in multi-walled carbon nanotube (MWCNT)/silica composite material. The measurement of OCP using potentiostat showed a linear range from 2.18×10^{-5} to 1.07×10^{-3} M urea concentration.

In the present experimental study, use of urea sensing film formed by encapsulated enzymes and the potentiometric response of the modified gold electrode (Urs/Alginate/Au) depends on encapsulated urease which forms redox active species which may undergo redox potential

In order to determine the response of fabricated working electrode in presence of different concentration of urea, we added 100µL of standard urea solution of varying concentration into the electrochemical cell and formation of ammonium and bicarbonate ions were monitored by measuring OCP with time till steady state value was attained for working electrode modified with urease encapsulated alginate nanogels cross-linked with different cations (Ca^{+2}, Sr^{+2}, Ba_{+2} and Mn^{2+}, Fe^{2+}, Co^{2+}, Cu^{2+}). A Nernst plot can be obtained by plotting the OCP vs log [Urea].


(A)
Potential difference (V)
-0.1540
-0.1545
-0.1550
-0.1555
-0.1560
-0.1565
-0.1570
-0.1575
-0.1580
0
10
20
30
40
50
60
70
80
90
100
Time (sec)


(B)
Potential difference (V)
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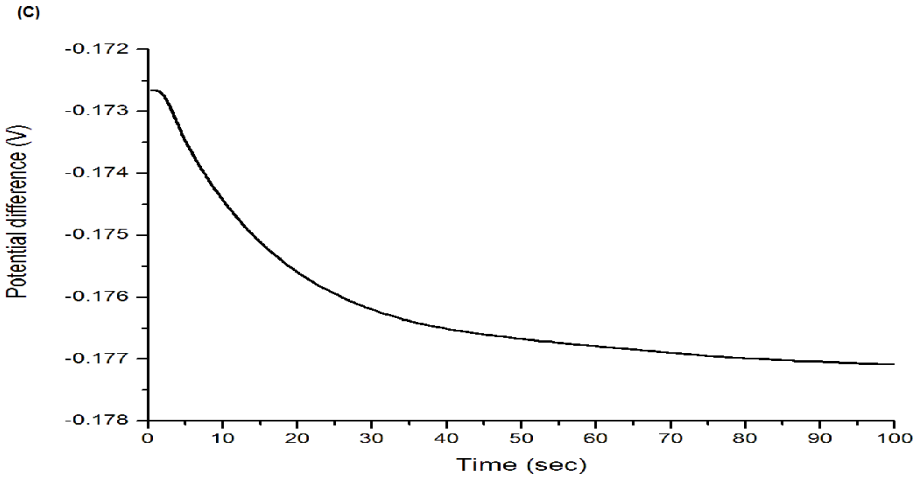
-0.16
0
100
200
300
400
500
Time (sec)


(C)
Potential difference (V)
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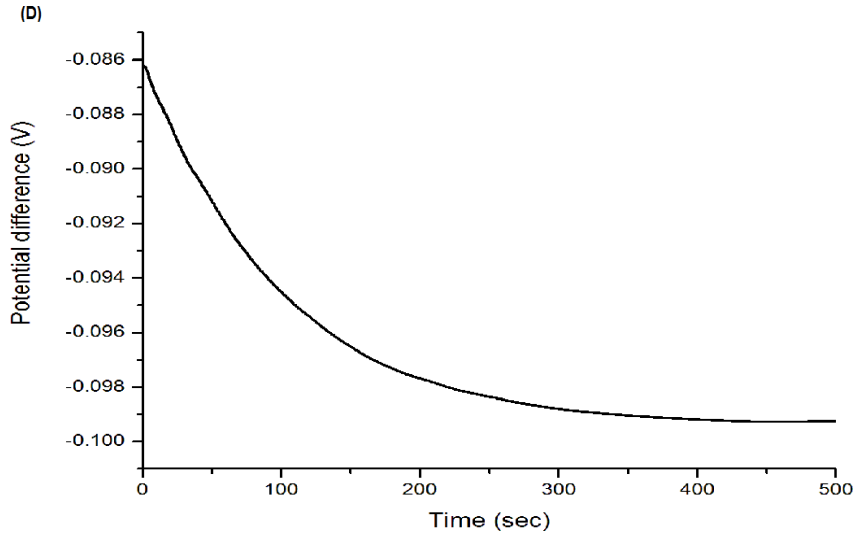
40
50
60
70
80
90
100
Time (sec)


(D)
Potential difference (V)
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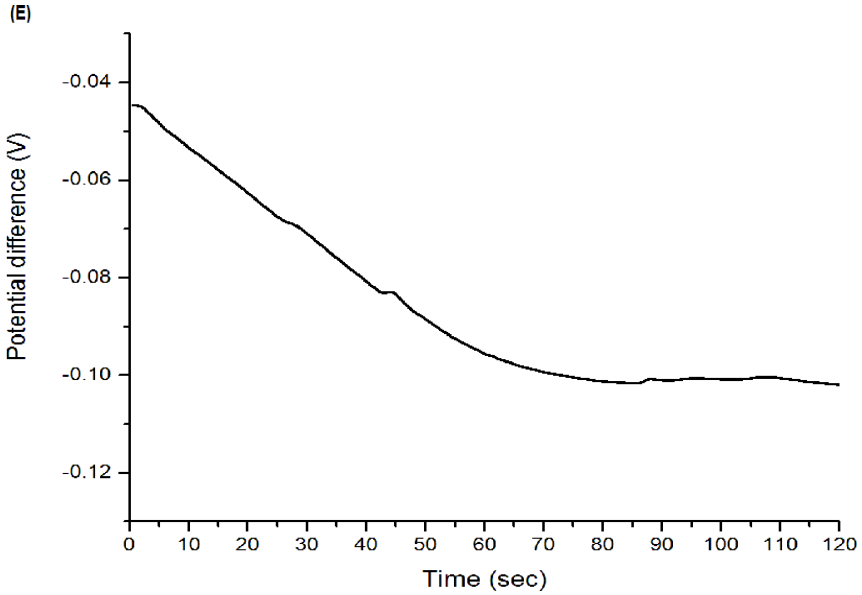
0
100
200
300
400
500
Time (sec)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Potential difference (V)
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60
70
80
90
100
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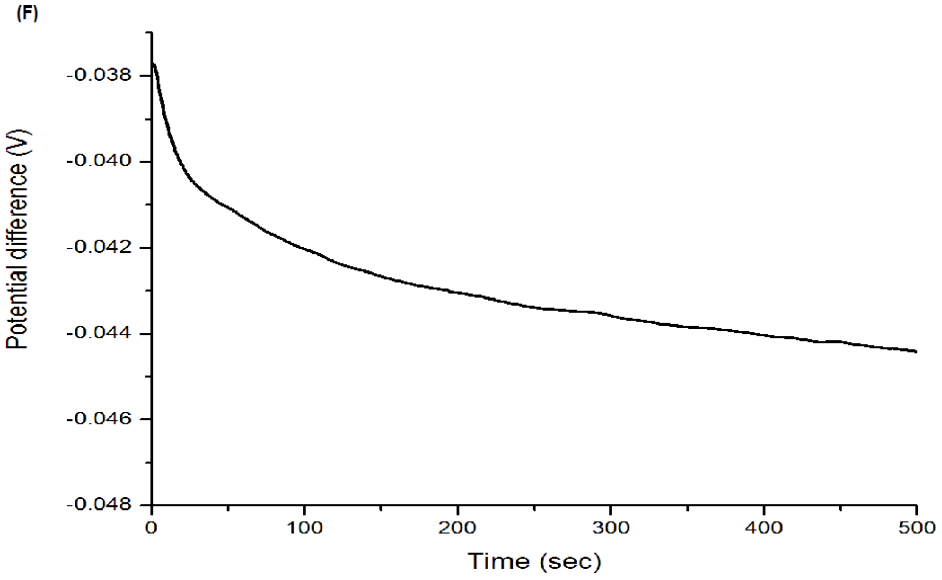
Time (sec)


(F)
Potential difference (V)
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200
300
400
500
Time (sec)

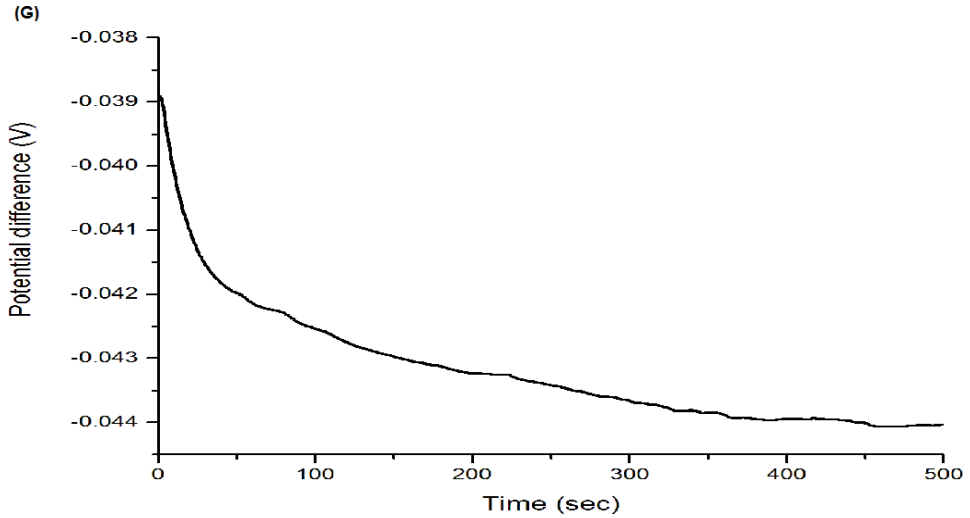
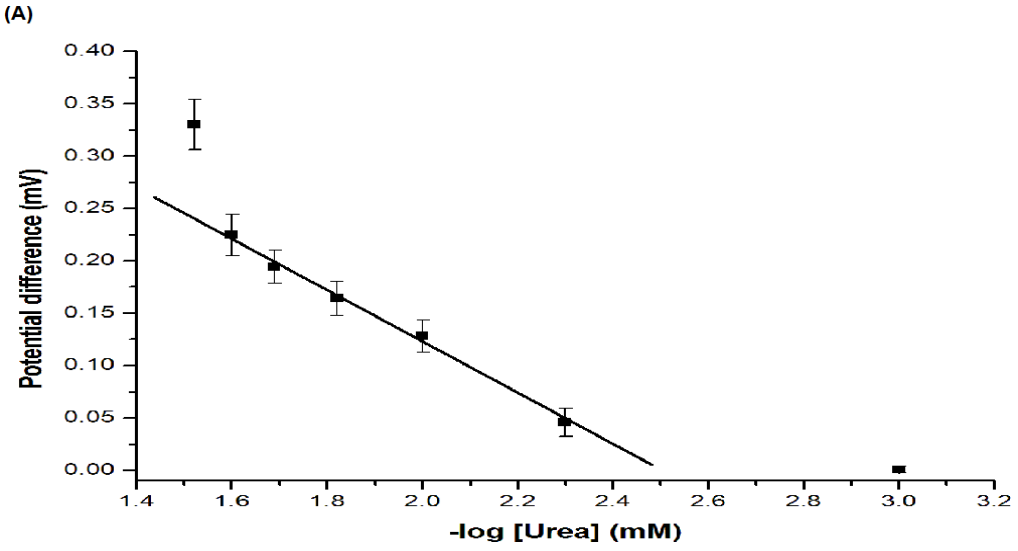
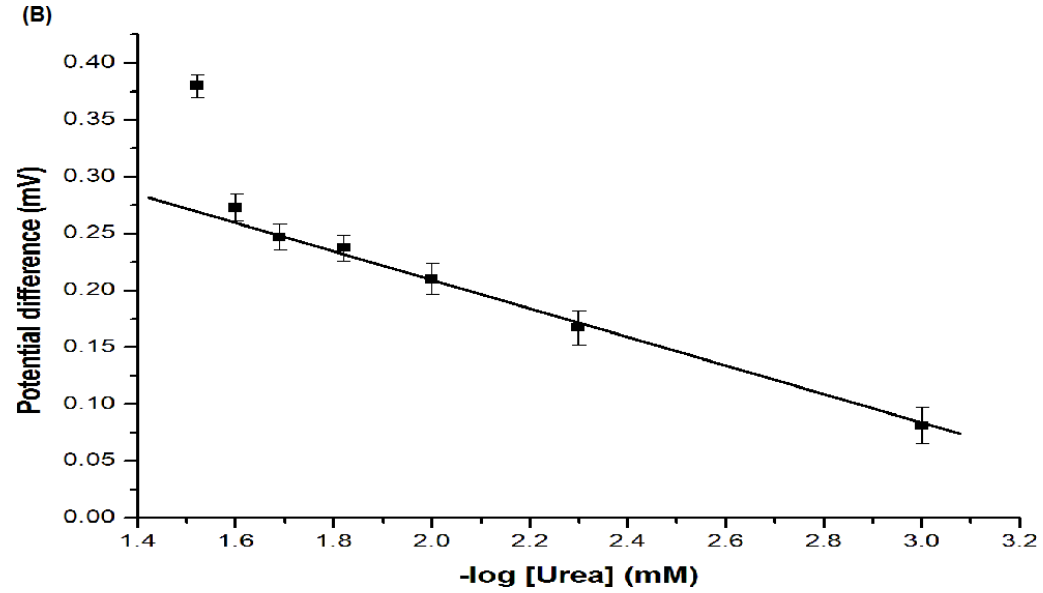


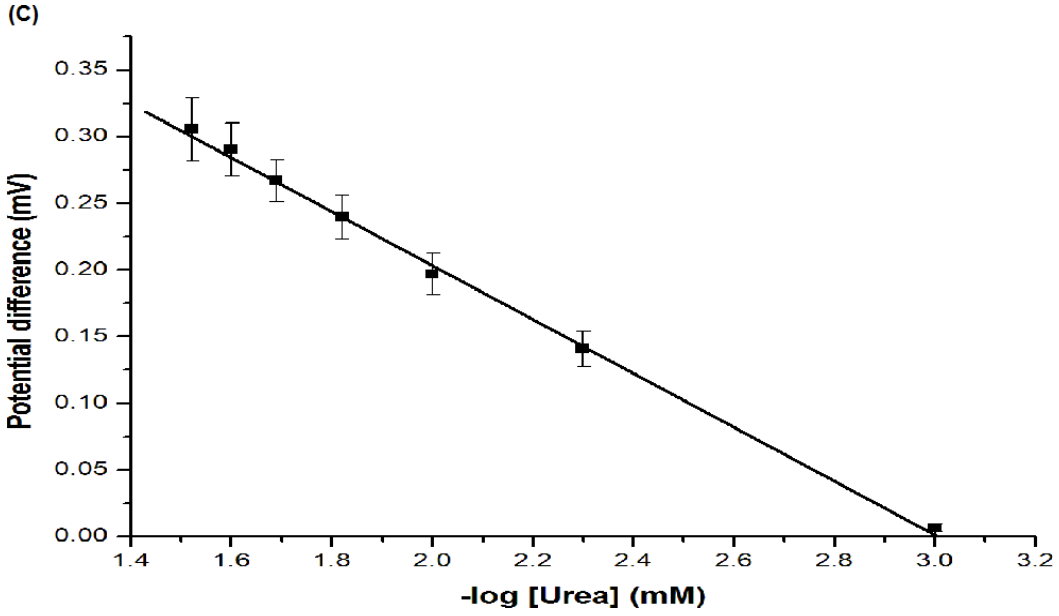
Figure: Response-Time curve showing variation of OCP with time in the presence of 1mM urea solution by using modified working electrode (Urs/ alginate/Au-Nano) with: Ca^{+2}, (B) Sr^{+2}, (C) Ba^{+2}, (D) Mn^{+2}, (E) Fe^{+2}, (F) Co^{+2}, (G) Cu^{+2} as cross-linking cations in alginate.

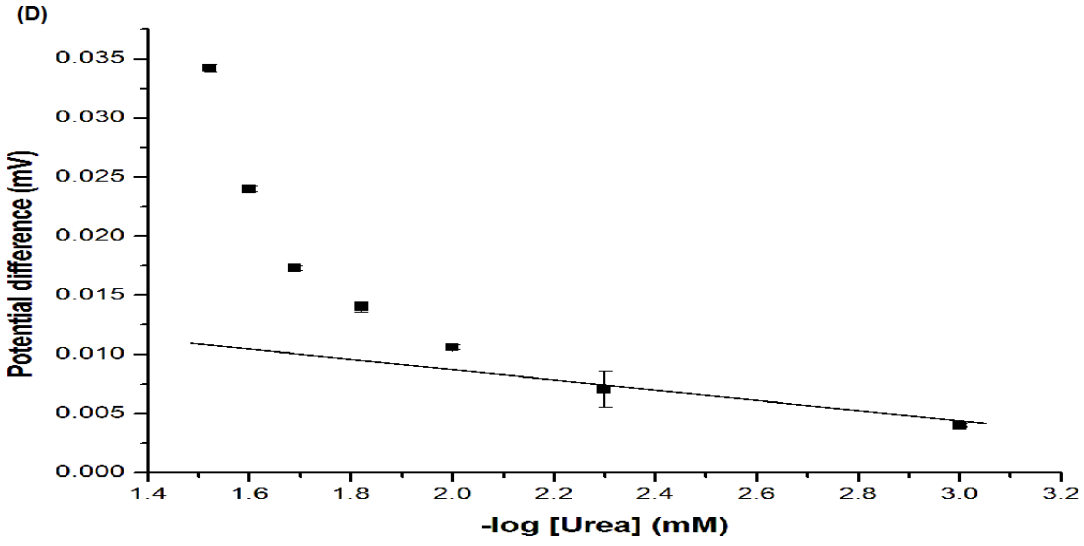
Response-Time curve obtained as potentiometric response for 1mM urea solution shows that time required to reach steady state potential i.e. the response time is different for different urease encapsulated nanogels and the potential attained during the response time measurement also show variation for the various working electrode (Urs/Alginate/Au-Nano) formed by alginate nanogels cross-linked with different cations. A comparison of response time of different working electrode containing nanogels cross-linked with Ca^{+2}, Sr^{+2}, Ba^{+2} shows that Ca/Ba-alginate nanogel based electrodes have fastest response time of about 1 minute while Sr-alginate based electrodes show stable OCP signals at ~4 minutes. Similarly, different working electrode containing nanogels cross-linked with Mn^{+2}, Fe^{+2}, Co^{+2}, Cu^{+2} shows that Fe^{+2} has fastest response (~1.5 minutes) towards analyte–urea, in comparison to Mn^{+2}, Co^{+2}, Cu^{+2} alginate based electrode (~7 minutes). As measurements are performed in a well stirred analyte free solution, at equilibrium condition where diffusion is the major process of mass transport, response time is governed by rate of analyte diffusion to the receptor site, i.e. towards immobilized urease in 3D nanogel structure or rate of urea-urease interaction or both. Thus, urea sensitive film formed by 3-dimensional structure of nanogels cross-linked with different cations is attributable to fast and slow response towards urea solution (analyte) which could affect the transport property of urea and its interaction with encapsulated urease.

Similarly, potentiometric response was measured for higher urea concentration (1-30mM) which covers the range of blood serum urea measurement in healthy humans and dialysis patients. A plot of potential difference between the initial and steady state value of OCP obtained with different concentrations of urea was used to develop calibration graph. The electrode to electrode reproducibility of six replicate working electrode was carried out in 1.0mM urea and standard deviation was calculated.




(B)
Potential difference (mV)
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
-log [Urea] (mM)


(C)
Potential difference (mV)
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
-log [Urea] (mM)


(D)
Potential difference (mV)
0.035
0.030
0.025
0.020
0.015
0.010
0.005
0.000
1.4
1.6
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
-log [Urea] (mM)

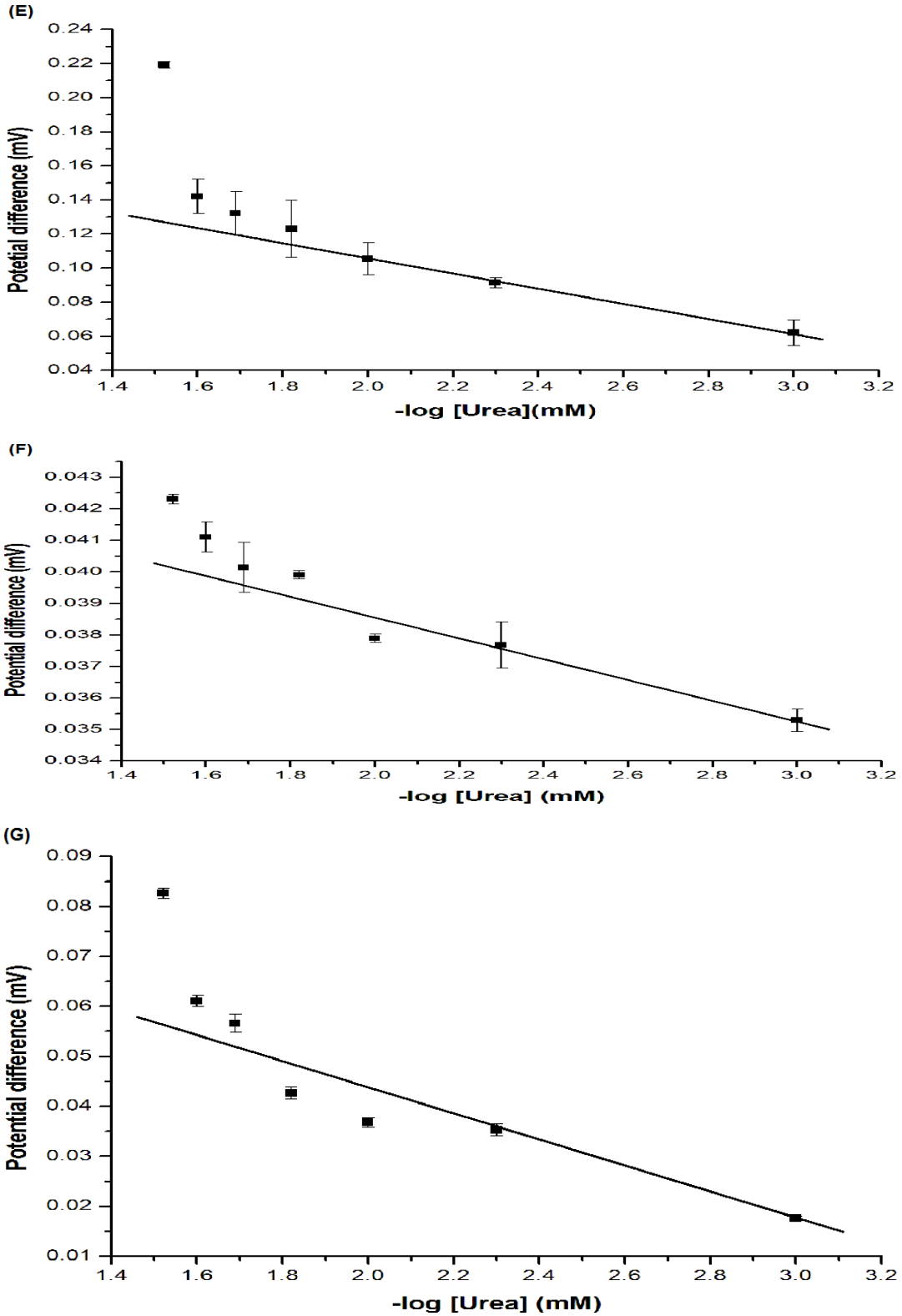


Figure: Calibration curve obtained by measuring potentiometric responses with different urea concentrations using modified working electrode (Urs/Alginate/Nano-Au) with:(A) Ca^{+2}, (B) Sr^{+2}, (C) Ba^{+2}, (D) Mn^{+2}, (E) Fe^{+2}, (F) Co^{+2}, (G) Cu^{+2} as cross-linking cations in alginate.

The calibration plot shows the linear range of urea detection by the application of modified working electrode (Urs/Alginate/Au-Nano). Sensitivity of each type of modified working electrode was measured as the slope of calibration curve. Maximum linear range for urea detection was obtained for Ba-alginate is similar to photometric enzyme kinetics. The different parameters optimized to determine the analytical performance of various modified working electrode.

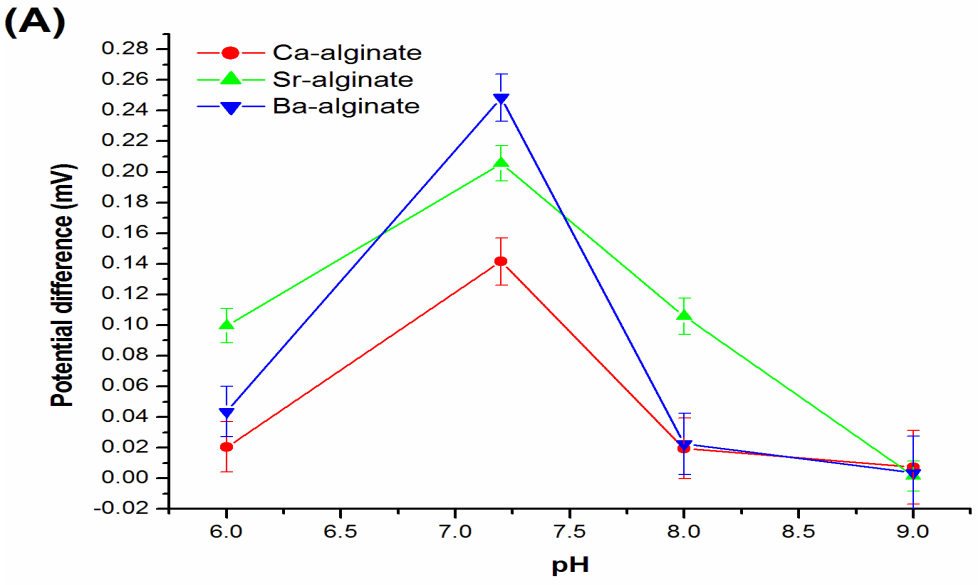
Table: Different analytical parameters showing performance of biosensor in 100 mM Tris-acetate buffer-saline measured with different urea concentrations using modified working electrode (Urs/Alginate/Nano-Au) with Ca^{+2}, Sr^{+2}, Ba^{+2} and Mn^{+2}, Fe^{+2}, Co^{+2}, Cu^{+2} as crosslinking cations in alginate.

S. No.	Type of alginate nanogels used in working electrode fabrication	Linear range of detection (Mm)	Sensitivity ΔV(mv) per Mm urea solution	Response time (sec)
1.	Ca-alginate	5-25	26.72	80
2.	Sr-alginate	1-20	17.1	240
3.	Ba-alginate	1-30	33.6	60
4.	Mn-alginate	1-10	0.8	450
5.	Fe-alginate	1-15	8.3	90
6.	Co-alginate	1-10	0.5	400
7.	Cu-alginate	1-10	1.1	400

Assuming that the urease concentration inside the different nanogels is constant, for the same concentration of urea in bulk solution, potentiometric response is different. The small steady state OCP changes translated into a calibration of lower slope and sensitivity were observed in the case of alginate nanogels cross-linked with the 3d metallic ions (Mn^{+2},Fe^{+2},Co^{+2},Cu^{+2}). These drastically small changes could be present due to internal diffusion resistance found in encapsulated enzymes in charged matrix. A comparison of modified working electrodes formed by alginate nanogel film cross-linked with alkaline earth metals shows best sensitivity with Ba-alginate gel while Sr-alginate showed least sensitivity and linear range of detection. These results supports the data obtained from photometric measurement.

Effect of pH

The effect of pH of the medium has considerable effect on the response of enzyme based biosensor as it controls the catalytic property of enzyme as well as enzyme stability in a matrix, therefore, it is necessary to optimize the optimum pH for the present modified working electrodes (Urs/Alginate/Nano-Au) cross-linked with different cations. The response of the electrode has been studied within the maximum urease activity range i.e. from pH 6–9 pH dependent variation measured as the magnitude of potentiometric response with l0mM urea solution at 25±1˚C. The maximum biosensor response in terms of potential difference was obtained at 7.2 pH. This pH optima correspond to the optimum pH of encapsulated urease enzyme in alginate nanogels. But itis different from value reported in MSDS from Sigma chemical Co. (i.e.7.4) as they have used Tris-HCl. Howell, S.F., Sumner, J.B., have studied the effect of different buffers on urease activity and concluded that activity of enzyme depends on the type of buffer, temperature and salt concentration. Enzymes are polyionic in nature and the charge property of proteins are governed by type of buffer, salt and temperature as it affects the charge distribution and protein structure. Thus, in the present study we studied the pH effect of Tris-acetate-saline bufferfor urease activity measurement which may be influencing the pH activity behavior of immobilized enzyme in polyelectrolyte alginate nanogels cross-linked with different ions.



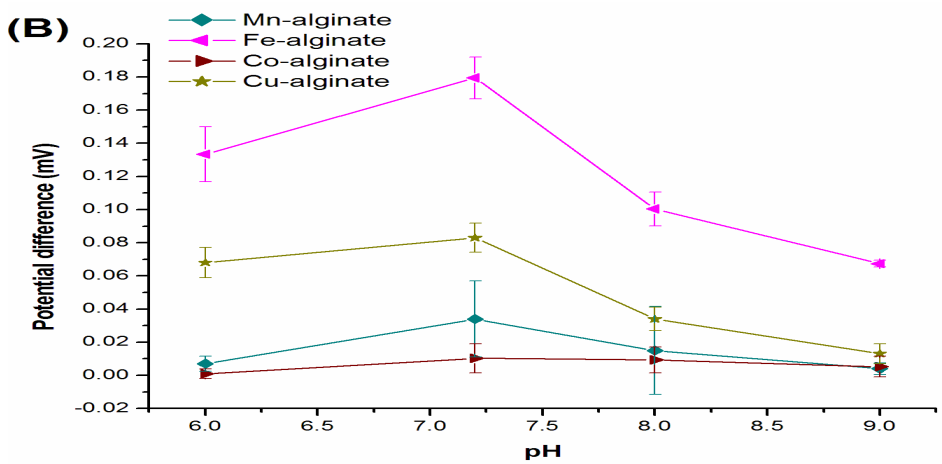


Figure: Effect of different pH on the potentiometric responses measured in 100 mM Tris-acetate buffer-saline with 10 mM of urea concentration using modified working electrode (Urs/Alginate/Nano-Au) cross-linked with (A) Ca^{+2} (—●—), Sr^{+2} (—▲—), Ba^{+2} (—▼—) and (B) Mn^{+2} (—◆—), Fe^{+2} (—◄—), Co^{+2} (—►—) Cu^{+2} (—★—) as cross-linking cations in alginate.

Effect of buffer-saline concentration on potentiometric response

pH is the most important variable which affect enzyme behavior by increasing or decreasing the H^+ ion concentration near the microenvironment of enzyme molecules enclosed in isolated compartment by enzyme encapsulation method. However, it is reported that ionic strength of the medium also affects the enzyme behavior at the values above 100mM. Thus, the influence of Tris acetate buffer-saline concentration on the response of the biosensor was further studied by calibrating the modified working electrode (Urs/Alginate/Nano-Au) cross-linked with different cations for different concentrations of buffer solution i.e. 50, 100, 300 and 500mM at constant pH~7.2. A comparison of the potentiometric response obtained for the enzyme coated electrode. The data suggest that the response for 10mM urea solution determined in the Tris acetate–saline buffer in the concentration ranges 50–100mM is comparatively higher where as the response of the enzyme-coated electrode measured above 100mM buffer concentration was considerably small. The difference in biosensor response induced by change in ionic strength of the medium may be attributed to the limited capacity at lower concentration of Tris acetate buffer to allow the local pH change within the enzyme coated membrane. This type of local pH effect in potentiometric urea sensor has also been observed by Mascini and Guibault group. Therefore, on the basis of effect of buffer concentration observed in our study, we have used 100mM, Tris acetate buffer at pH~7.2 for all the measurements.

Table A: Effect of buffer concentration (Tris-acetate-saline, pH 7.2) on sensitivity of potentiometric responses measured with 10mM urea concentrations using modified working electrode (Urs/Alginate/Nano-Au) with Ca^{+2}, Sr^{+2}, Ba^{+2} as cross-linking cations in alginate.

Concentration of buffer (mM)	Sensitivity of Ca-alginate based electrode (mv per mM urea)	Sensitivity of Sr-alginate based electrode (mv per mM urea)	Sensitivity of Ba-alginate based electrode (mv per mM urea)
50	29.6	28.4	48.2
100	24.2	22.1	34.0
300	9.3	6.1	8.9
500	4.5	1.0	5.2

Table: Effect of buffer concentration (Tris acetate saline, pH 7.2) on sensitivity of potentiometric responses measured with 10 mM urea concentrations using modified working electrode (Urs/Alginate/Nano-Au) with Mn^{+2}, Fe^{+2}, Co^{+2}, Cu^{+2} as cross-linking cations in alginate.

Concentration of buffer (mM)	Sensitivity of Mn-alginate based electrode (mv per mM urea)	Sensitivity ofFe-Alginate based electrode (mv per mM urea)	Sensitivity of Co-alginate based electrode (mv per mM urea)	Sensitivity of Cu-alginate based electrode(mv per mM urea)
50	8.6	14.4	0.8	6.6
100	6.3	9.8	0.5	4.4
300	09	09	0.01	1.3
500	04	09	0.00	0.3

CONCLUSIONS

In the present study, we have tried to develop a bioreceptor using urease enzyme encapsulated in polyelectrolyte film of alginate based nanogels cross-linked with different divalent cation for the detection and measurement of urea in blood serum. Gold nanoparticles based electrode surfaces were modified by drop-casting the urease encapsulated alginate nanogel suspension and used as working electrode for urea determination in three electrode based electrochemical measurement system. Surface morphology of the various alginate nanogel based films were studied by SEM, suggesting Ba-alginate nanogels based modified electrode is more granular and porous. It may provides larger surface area for enzyme–substrate catalytic reaction and hence advantageous for electrochemical measurement in heterogeneous systems. In the present work, the urea sensing film formed by encapsulated enzymes show potentiometric response of the modified gold electrode (Urs/Alginate/Nano-Au) that depends on the mass transportation forming electro-active species

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