ISSN: 0975-3583,0976-2833 VOL8, ISSUE 2, 2017

EXPLOITATION OF SURFACTANT SELF-ASSEMBLY FOR PREPARING UREASE ENCAPSULATED ALGINATE NANOGELS

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Abstract

In biomedical applications, alginate nanoparticles and micro particles cross - linked with Ca+2 particles is widespread. We utilise microemulsion polymerization to make alginate nanoparticles (nanogels) with various linking ions (Sr+2, Ba+2, Ca+2) to encapsulating urease enzyme as a model protein (jackbeans). Dispersion droplets demonstrated good stability and narrow, monomodal ranges with radii of 65 ± 10 nm when alginate quantities in the aqueous phase were 0.2 wt percent. The size of the nanogel is determined by the linking cation's affinity for the straight alginate chain's mannuronate and guluronate time in deciding units. Spectrometer X-ray spectroscopy, dynamic light bouncing ensemble particle radii of nano alginate particles, scanning electron microscopy and zeta potential were characterize the surface charge and physical stability of the nanogels. Ba-alginate can be utilised as an alternative matrix for nano-encapsulation of molecules and in biological applications, according to this study and its shows better stability and high surface charge . Generally divalent cations are cross-linked for the development of suitable alginate gels for different applications like BUN kit development ,therapeutics.

1.Introduction

Enzyme-based electrochemical biosensors account for the vast majority of biosensors built and sold for diverse purposes. In enzyme-based biosensors, an enzyme is either attached to the electrolyte interface, as in a phase biosensor, or an enzyme processor is linked to the probe by a stream line, as in a Flow Injection Analysis (FIA) system. Biosensors including oxido-reductases, hydrolases, and lyases have been developed for medical applications [1], pharmaceutical applications [2], food applications [3], environmental monitoring applications [4], and military applications [5]. Biosensors based on enzymes can be used to monitor a variety of substrates. Although producing operationally reliable biosensors for long-term use in monitoring systems is one of the primary aims of biosensor research [6], enzyme-based biosensor growth is restricted by their lower viscosity. As a result, enzyme stability is an essential issue for a variety of biomedical application. Normalization of enzymes can take the form of a lower inactivation rate constant,

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resulting in a longer half-life, or resistance to inactivation by chemicals like thiourea and guanidine hydrochloride (GdmCl), or exposed to new pH and temperature conditions. Hydrogen bonds, hydrophobic forces, dispersion forces, Disulphide bonds, and ionic contacts are some of the connections that impact protein stability. External substances can be used to influence these parameters, which can help to improve enzyme stability [7]. Many various approaches, such as protein engineering [8], using enzymes from naturally partially purified microorganisms [9-10], immobilization of enzymes in appropriate matrices [11], and adding stabilising chemicals to enzymes [12-13], can increase enzyme stability.

Binding the catalyst molecule on a solid substrate is essential for increasing the stability of enzymes for electrochemical biosensor applications. It reduces protein unfolding, making the protein more stable. It has been observed [14] that matching surfaces between the catalyst and the substrate are more stable when there is a good connection in the immobilized enzymes. in addition to multipoint attachment through cross-linkages; improve the stability of immobilized enzymes. As a outcome, immobilization has a important influence in influencing enzyme activity and, as a result, can significantly add to the biosensor's sensitivity. The construction of a conductive gel micro-sensor for biomedical applications [19] has been made possible by advances in microfabrication methods [15], biosensor technology [16], and gel-material development [17-18]. Alginates, a naturally present biopolymer, are good candidates for biosensor manufacturing because they create stronger gels with divalent or trivalent cations under relatively moderate circumstances, are moisture, and maintain the biological activity of sensitivity molecules [20]. By pouring an alkaline phase of alginate into yet another solution containing calcium ions, macroscopic calcium alginate beads (13 mm) may be easily generated [21]. Various enzymes have been successfully isolated [22-24], implying that hydrophilic alginate microgels may encapsulate protein in a 3-dimensional microstructure with shields in a predetermined volume and appropriate micro - environment. Nanomaterials in biosensors, on the other hand, have advantages over traditional techniques in terms of compassion, resilience, responsiveness, signal-to-noise ratio and wide surface area for high enzyme loading [25]. However, preparing smaller alginate gel particles (nanogels) is more challenging.

We used nano emulsion technology to investigate the abstraction of urease enzyme in alginate nanogels cross-linked with different divalent cations by sol-gel transformation, because it provides

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an extremely choosy reply to the significant biological particle urea, which is of significant concern i) in medical analysis, as it is the main consistent catalog of renal utility [26]; ii) in external scanning, since it is employed as a nitrogenous fertilizer that produces physical degradation [27]; and iii) in the identification of corruption in Cadbury or the examination of the nursing dairy cows' nutritional programme [28].

This immobilization approach offers the features of microemulsion technology to produce alginate-based nano-droplets with alginate's crystallisation ability to encapsulate urease in a benign, nontoxic environment with better enzyme activity preservation. To establish the optimum surrounding substance for the construction of urea biosensors, researchers evaluated various immobilization characteristics, enzyme kinetics, reusability, storage, and stability tests in alginate nanogels cross-linked with several divalent cat ions (Sr^{+2}, Ba^{+2}, Ca^{+2} and Co^{+2}, Fe^{+2}, Cu^{+2}, Mn^{+2}).

2.Review of Literature

The creation of bio-receptors on the transducer surface is primarily accomplished using immobilised enzyme in the manufacturing of enzyme-based electrochemical biosensors. However, because proteins are fragile, immobilizing them on a solid substrate without impairing their bioactivity is challenging. Immobilization of enzyme in nanostructure material may therefore be utilized to concentrate and preserve active substances in a specified volume, resulting in a divided micro - environment with adjustable characteristics that can be used to regulate dispersion and reactivity as needed for the sensor function [31].

Proteins, especially enzymes, need a liquid condition to preserve their 3d structure for biological function, unlike nucleic acids, which may be kept in dry environments for an extended period [32]. As a result, the immobilization of the urease enzyme in alginate nanogels has been investigated.

According to a literature review [22-23, 33], the size of alginate particles produced by the gelatin technique rises as the quantity of alginate sol improves. The impact of alginate solution on the formation of swelling revere micelles was investigated in our work described in Chapter 2, and it was discovered that 0.2 percent alginate sol (w/v) was effective. It’s appropriate for the creation of urease encapsulated alginate nanocrystals. With a size of around 100nm that are cross-linked with various cations (Sr^{+2}, Ba^{+2}, Ca^{+2} and Fe^{+2}, Co^{+2}, Mn^{+2}, Cu^{+2}). As a result, various

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concentrations of lyophilized urease were pre-mixed with 0.2 percent alginate sol to create urease encapsulating alginate nanogels for urease immobilization in alginate nanogels.

A part of the protein is immobilized in the 3-dimensional structure of the alginate gel after urease encapsulation by sol-gel transfer, while the remainder remains unsecured. To evaluate various enzyme immobilization parameters, enzyme tests and protein estimates were performed. Enzyme loading efficiency and percent immobilization are essential metrics for describing the efficacy of enzyme immobilization inside a solid support because they provide information about the immobilized enzyme's activity. We can anticipate the activity per unit mass of connections in the solid support by comparing these characteristics.

3.Proposed Methodology Setup

3.1Basic resources

Urease, Sodium alginate (mol. wt. 120,000–190,000) were purchased from Sigma-Aldrich. Polyoxyethylenesorbitan, calcium chloride, mono-oleate (Tween®80), strontium chloride, barium chloride, manganese chloride, ferrous chloride, cobaltous chloride, copper chloride, Nessler’s reagent , sodium chloride and urea were supplied by Fisher Scientific. CDH provided the hexane. All of the compounds were analytical grade (AR) and were utilized exactly as they were. Throughout the trials, triple distilled water was utilised.

3.2Alginate nanogels of urease: Encapsulation

Different cations (Mn+2, Cu+2, Fe+2, Ca+2, Sr+2, Ba+2, Co+2) were used to crosslink alginate nanogels. Here the encapsulation is done for biomolecules by using mild methods to produce particles in desired size,the process for encapsulating urease enzyme was similar to that described above, with the exception that the lyophilized urease was combined with the alginate solution ahead of time (i.e alginate sol). The nanogels were cleaned, lyophilized, and reassembled in 100 mM Tris acetic buffer-saline (pH 7.2) for further usage. Crosslinking cation and its affinity vary the size of the nanogel.

3.5Evaluation of enzyme

The relevant enzyme activity of soluble urease, enclosed urease in alginate nanogels inter with various cations (Ba+2, Ca+2, Sr+2 and Co+2, Cu+2, Mn+2, Fe+2) (i.e. immobilized urease), and left over enzyme in wash alternative were all resolute separately using a spectrophotometric assay

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method as follows: The immobilized urease nanogels (0.001 g) or a fixed volume of standard solution or rinse solution containing a permeable enzyme urease (0.1mL) were incubated in 0.1M urea with occasional shivering. The quantity of NH3 released was measured using Nessler's reagent [22] after incubation for a certain time period of 30 min. At 405nm, the absorbance of the samples with a spectrophotometer (Ocean optics UV-Vis spectrophotometer, Model dH-mini). Under normal test surroundings (100mM Tris-acetate-saline buffer, pH 7.2, and 25°C), one part of urease movement relieves 1mol of NH3 from 0.1 M urea per minute.

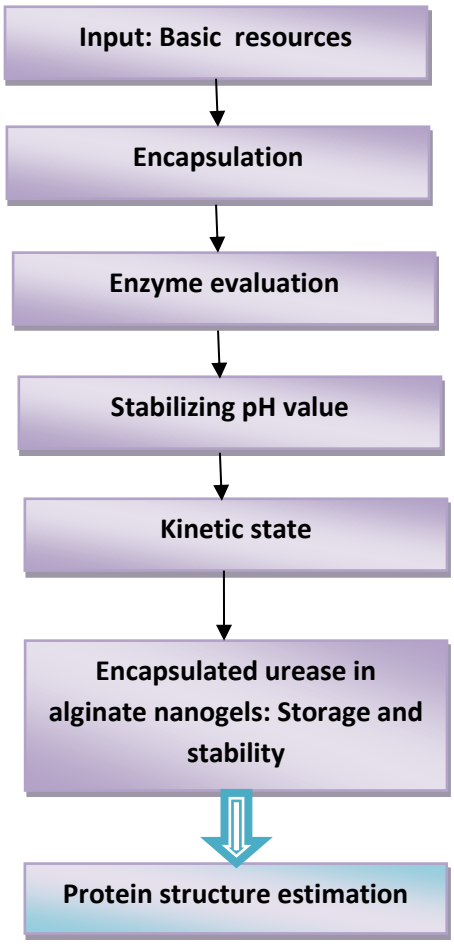

Figure 1: Steps of urease encapsulated alginate nanogels preparation

The protein content of the alginate nanogel was assess using Pignolet, L.H., L., Govindarajoo, G., Nowick, J.S.'s Waldman, A.S., Schechinger technique of de-cross-linking alginate nanogels in

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brine solution [29]. In a nutshell, lyophilized alginate nanogels were distributed in a saturated NaCl solution of 10 mL. At ambient temperature, the above mentioned combination was agitated for 12 hours in a shaker (160 RPM). The clear solution was then filtered further using a syringe filter. Bradford's technique [30] was used to evaluate the protein content of the alginate nanogels from the filtrate, which was released while breaking cation alginate crosslinks. The Bradford technique of protein quantification was also used to assess the wash solution's remaining protein content [30]. The alginate nanogel containing different cross link ions were suspended in water and slowly placed in standard cell to avoid air bubbles, small white pellets of alginate nanogels were obtained. The following equations were used to determine the enzyme loading efficiency and capacity of the nanogels:

$$A.\ Enzyme\ loading\ efficiency = \frac{Wt.of urease\ in\ alginate\ sol - Wt.of\ residual\ urease}{Wt.of\ urease\ in\ alginate\ sol} \times 100 \quad \text{----(1)}$$

$$B.\ Loading\ Capacity = \frac{Wt.of\ urease\ in\ alginate\ sol - Wt.of\ residual\ urease}{Polymer\ weight} \quad \text{-----(2)}$$

The enzyme activity retention in the alginate nanogel was calculated as percent immobilization:

$$\text{C. Percent immobilization (\%)} = \frac{\text{Specific activity of immobilized urease}}{\text{Specific activity of soluble urease}} \times 100 \quad \text{------(3)}$$

As previously stated, the specific activity of immobilized urease was determined. By subtracting the specific activity of urease during washing (unbound urease) from the specific activity of total soluble enzyme, the specific activity of soluble urease was determined.

3.6 Stability studies about pH

In a pH range of 5.2-10.2 in 100mM Tris acetate buffer, the influence of pH on the enzyme concentration of soluble and enclose urease in alginate-based nanogels that have been dye-cross linked (Sr+2, Ba+2 ,Ca+2and Cu+2, Mn+2, Fe+2, Co+2) was investigated. In the Tris acetate-saline buffer, a sufficient measure of soluble urease and lyophilized immobilized urease were incubated for 30 minutes, and enzyme activity was measured as described in the Enzyme Assay.

3.7 Kinetics steady-state

At 251°C, the influence of flow rate on urea hydrolysis was studied by changing the urea amount from 1 to 30 mM at the optimal pH of 7.2 for soluble and immobilized enzyme. The enzyme activity assay was performed based on the current Enzyme Assay guidelines. The Michalis-Menten

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constant (Km) and the upper limit enzymatic reaction velocity (Vmax) were estimated using the Lineweaver–Burk plot and turnover number (K_{cat}) was calculated.

3.8 Encapsulated urease in alginate nanogels: Storage and stability

The solubility and encapsulating immobilized urease in alginate nanogels were kept at pH 7.2 and 4 C in 100mM of Tris acetic saline buffer. Under identical circumstances, the activity of stored urease (immobilized and soluble) was carried out and the results at regular intervals for four weeks using the assay methods described above. The duration was plotted against the % residual activity. A figure like this was used to evaluate electrocuted urease enzyme durability in different nanogels with 90% residual enzyme activity

3.9 Estimating protein structure

The protein content of urease-encapsulated alginate nanogels border with various divalent ions (Ba+2 , Ca+2, Sr+2 and Mn+2, Cu+2, Fe+2, Co+2) was measured using A.S., Schechinger, G., Nowick, L., Govindarajoo, J.S.'s Pignolet, L.H., Waldman, technique of de-cross-linking alginate nanogels . In a nutshell, 2 mg of lyophilized urease encapsulated alginate nanogels cross-linked with various divalent ions (Sr+2, Ba+2, Ca+2 and Co+2, Mn+2, Fe+2, Cu+2) were put to a test-tube and add 10 ml of saturated NaCl solution and allowed to shake (160 RPM) at ambient temperature for 12 hours. The divalent cation disrupts the cross-links established between the linear alginate strands, releasing the urease protein. The turbid solution was then filtered, and the filtrate was used to determine protein concentration.

Bradford's technique [30] was used to calculate the amount of urease protein. The Bradford technique of protein quantification was also used to determine the wash solution's remaining protein concentration [30].

4.Numerical result and discussion

4.1 Immobilization of urease enzyme in alginate nanogels: pH based stability studies

The charge distribution characteristic of enzymes changes with the pH of the bulk phase, making them zwitter-ionic in nature. The rate of enzymatic urea hydrolysis by immobilized urease enzyme varies depending on the medium's pH. As a result, it's crucial to investigate the effect of pH on immobilized urease enzyme production. Figures 2 and 3 illustrate the activity of inter urease enclosed in alginate nanogels with (Sr+2, Ba+2, Ca+2 and Cu+2, Mn+2, Fe+2, Co+2) within the maximal urease activity range (pH 5 to 10) using a 10 mM urea solution at 251°C. Figures 2 and

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3 demonstrate that the highest urease activity (percent) determined by enzyme assay technique was reached at pH 7.2. However, because they employed Tris-HCl to test pH optima, it differs from the value stated in the MSDS from Sigma Chemical Co. (i.e. 7.4). The impact of various buffers on urease activity was also examined by Howell, S.F., and Sumner, J.B. [38], who came to the conclusion that the movement of the enzyme is independent on the type of buffer, temperature, and salt content. Illanes, Andrés [35] reported on the impact of matrix on the pH maxima of several enzymes. The charge feature of proteins is regulated by the kind of buffer, salt, and temperature because it influences the charge distribution and protein expression. The pH influence of Tris-acetate-saline buffer on urease protein identification was investigated in this work. Varying metal ions cross-linked in alginate gels have little influence on the pH optima. Thus, in the current work, a pH-based microenvironment is critical for enzyme catalytic site activity, which leads to biochemical changes.

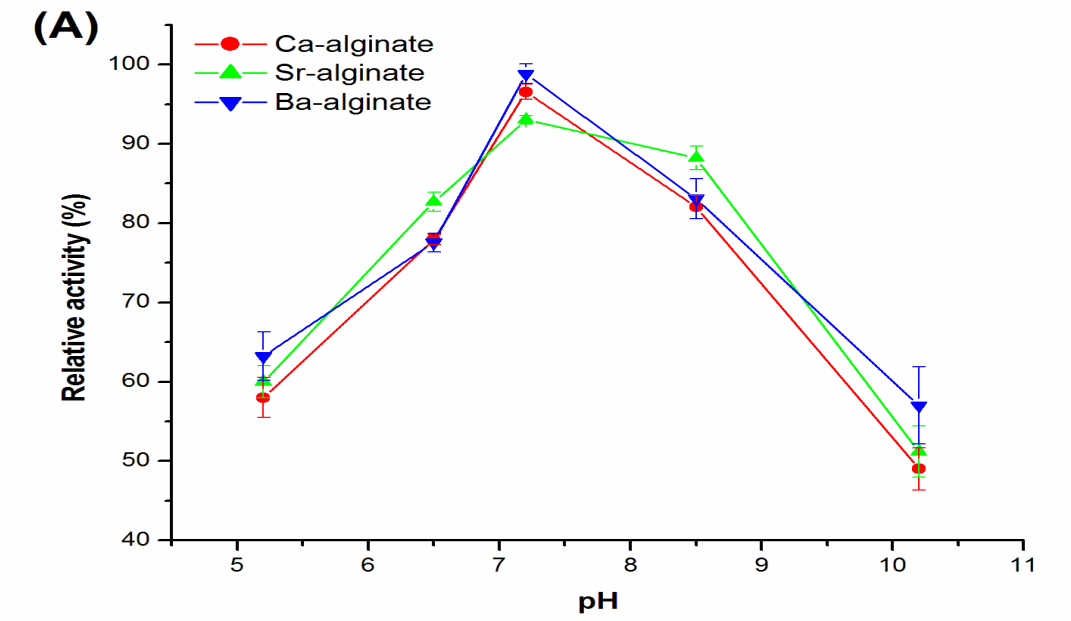

Figure 2:Variation of percent relative activity with pH studied for urease enzymes embedded in inter alginate nanogels Ca^{+2} (—●—),Sr^{+2} (—▲—), Ba^{+2}(—▼—).

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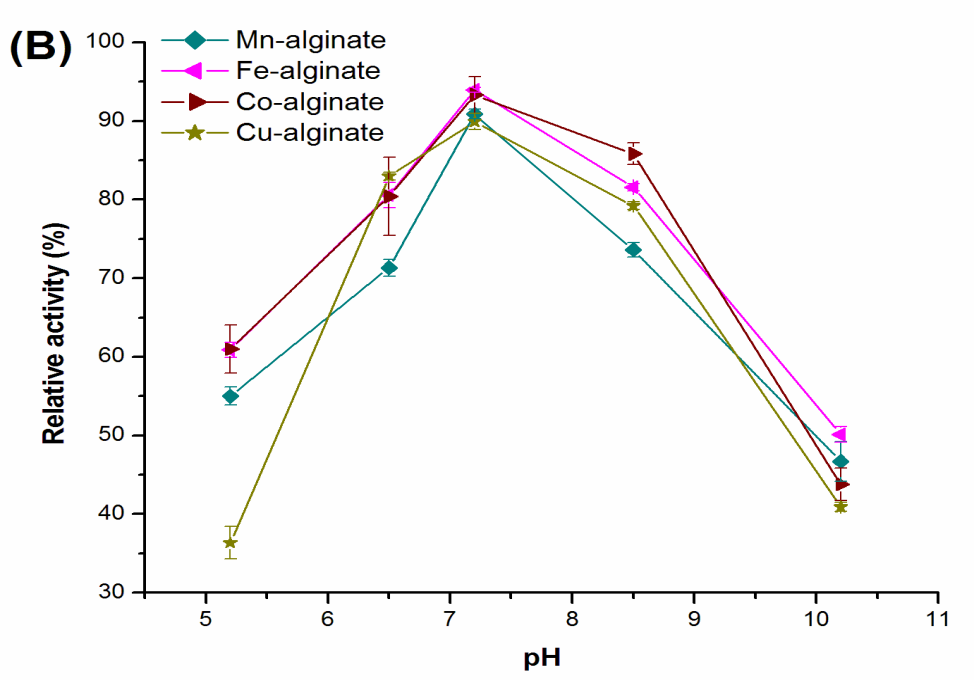

Figure 3: Variation of percent relative activity with pH studied for urease enzymes embedded in inter alginate nanogels with Mn^{+2} ( ), Fe^{+2} ( ), Co^{+2} ( ) Cu^{+2} ( ).

4.2 Kinetics steady-state results

The biocatalytic property of urease immobilized on a solid phase, such as alginate nanogels, is measured in a heterogeneous system in which the enzyme is in one step and the substrate/product is in the bulk aqueous phase, with the biochemical reaction occurring at the exterior or inside the nanoparticle (immobilized biocatalyst). As a result, substrate or product diffuses through the nanogel pores, potentially interfering with mass transfer. The following reaction rate [39] can be used to describe the enzyme hydrolysis of urea:

$$(NH_2)_2CO + 2H_2O \rightarrow 2NH_4^+ + CO_3^{2-} \quad (4)$$

Using Nessler's reagent [40] and assuming an extremely competitive mechanism for ammonia detection, the following rate expression emerges:

$$r = V_{max}.[S]/(K_M + [S])(1 + [P]/K_P) \quad (5)$$

where [S] and [P] denote the concentrations of substrate and ammonium ions, accordingly; Vmax denotes the maximal reaction rate; Km indicates the Michaelis-Menten ratio, and KP signifies the enzyme system's dissociation consistent. The initial rate technique was used to evaluate the kinetic data, and (Km) and Vmax values were derived using Line weaver-Burk plots for the various

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nanogels, as shown in Table 1. The average concentration of urea examined was 1-30mM (6 mg/dL to 180 mg/dL), which may be used to test blood serum urea in both healthy and sick people. The calibration curve for alginates gels produced with MnCl2, FeCl2, CoCl2, CuCl2 gelling solutions and CaCl2, SrCl2, BaCl2 (Calibration curve of encapsulated urease polymeric nanogel cross linked with Sr+2, Ba+2, Ca+2and Cu+2, Mn+2, Fe+2, Co+2) were obtained.

Table 1: Kinetic parameters of border urease enzyme encapsulated in various alginate nanogels with Sr^{+2}, Ba^{+2} ,Ca^{+2} and Co^{+2}, Mn^{+2}, Fe^{+2}, Cu^{+2}.

S.No.	Type of alginate nanogel containing urease	Vmax (mmol/min)	Km (mM)	Linear vary of calibration curve (mM)	Turn over no.
1.	Urease	--	3.4	--	--
2.	Ca-alg	2.42	0.45	2.0-24.0	59.424
3.	Sr-alg	1.72	0.67	5.0-19.0	70.82
4.	Ba-alg	3.01	1.16	0.8-30.0	92.8
5.	Mn-alg	1.12	2.59	5.0-15.0	49.42
6.	Fe-alg	1.6	0.31	5.0-25.0	80.52
7.	Co-alg	1.31	0.51	1.0-5.0	61.29
8.	Cu-alg	1.15	4.1	1.0-10.0	78.11

The Line weaver-Burk plot was created with an enzyme concentration of 0.05 mg/ml, which resulted in the highest percent immobilization for all of the nanogels tested. In a stable region where the enzyme is immobilized inside a solid support and the surface present in bulk solution conduct mass transfer, the nature of the double inverse plot is an indicative of enzyme-substrate interaction characteristics. It's tough to calculate the different parameters of mass transfer and strict hindrance. However, the nature of the plot in Sr-alginate, Mn-alginate, and Cu-alginate is non-linear, suggesting that validation limitations and steric hindrance in enzymatic interaction inside the gel matrix are possible. The linear form of double plot with Ca-alginate, Ba-alginate, Fe-alginate, and Co-alginate, on the other hand, suggests that carrier matrix is compatible in urea and expresses maximum activity with it [35]. Except for Mn-alginate and Cu-alginate, the Km value

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derived from the linear plot is lower than the Km value reported for soluble urease in all cases, showing that urease trapping inside the segmented, porous nanogels is favourable.

5.Conclusions

A moderate approach that uses surfactant self-assembly for templating was used to effectively produce urease encapsulated alginate nanogels of various sizes. In terms of turnover number, enzyme loading competence, loading capability and the immobilization capabilities of various nanogel enzyme encapsulating methods were evaluated. Thus, the protein-loaded Ba-alginate nanogels were the best at preserving enzyme catalytic activity during element creation, improvement, and storage in 100mM Tries-acetate buffer. Optical detection of urea has been described using a variety of nanoparticle immobilization matrices. For urea detection, the performance of a Ba-alginate nanogel, which surpasses the other nanogels, was examined. With 90% activity, ba-alginate may wrap the protein/enzyme for 20 days, significantly outperforming the Ca-alginate, Sr-alginate, and 3d metal alginate nanogels used in our research. Ba-alginate colloidal particles do not bundle and have a highest linear vary of urea uncovering due to their high surface blame. The ease with which enzymes may be immobilized in Ba-alginate nanogels, as well as their storage immovability, making them promising for diagnoses, BUN (blood urea nitrogen) kit growth, and therapy.

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ISSN: 0975-3583,0976-2833 VOL8, ISSUE 2, 2017