Covalent coupling of Urease on Acrylamide-co-2-hydroxy ethyl methaacrylate and Its use in urea hydrolysis by batch process.

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Abstract: We have immobilized urease on copolymer of acryl amide and 2-hydroxy ethyl methaacrylate by covalent coupling .. We have carried out covalent coupling by activating the amino group by glutaraldehyde and hydroxyl group by p-tolyl sulphonyl chloride and p-benzoquinone. We have optimized various covalent coupling conditions such as activation agent concentration, activation agent coupling time, enzyme loading , enzyme coupling time, and coupling pH. We have carried out activation of amino and hydroxyl group separately and have also carried out simultaneous activation of both groups and found some interesting results. We have further carried out the hydrolysis of urea by batch process using covalently bound enzyme.

Keywords: acrylamide-co-2-hydroxy ethyl methaacrylate,, urease, covalent coupling, urea hydrolysis, batch process.

1.0. Introduction: Enzymes are classified into six major classes .Urease (EC 3.5.1.5) belong to the class hydrolase . The CAS NO of Urease is 9002-13-5 . It carries out the hydrolysis of urea to give ammonia and carbon dioxide .

$(NH_2)_2CO + H_2O \longrightarrow CO_2+H_2O$

Urease is used mainly in biominerialiazation inspired process. Urea is found in environment and it is thought to promote eutrophication. We have used urease for hydrolysis of urea. Covalent coupling is a method of immobilization which is irreversible. Many researchers have carried out covalent coupling of

Urease on various supports. Polyacrylamide and its derivatives has been used by many co-workers for covalent coupling [1,2], on cellulose [3,4],on selective potentiometric membrane [5], on polysiloxane matrices containing 3-aminopropyl and 3-mercaptopropyl groups[6] ,On dialdehyde porous starch [7],Acryl amide-co-2-hydroxyethyl methaacrylate has been used for covalent coupling using Horseradish Peroxidase .[8], And also for covalent coupling of Pepsin [9]. In present work we have also carried out the covalent coupling on Acrylamide-co-2-hydroxyethylmethaacrylate using urease enzyme . Till date no reports have been found on covalent coupling of urease on this support . We have optimized various covalent coupling conditions using immobilized urease and used free and covalently bound urease on AAm-co-HEMA for urea hydrolysis using batch process and have optimized various reaction parmaters here also .

Chemicals:

Urease (200U/mg), Urea, Phenol, Hypochloride solution, Acryl Amide , 2-hydroxyethyl methaacrylate , p-tolyl sulphonyl chloride , p-benzoquinone.

2.0.Experimental:

2.1.Preparation of Copolymer :

We have prepared the copolymer of acrylamide and 2-hydroxy ethyl methaacrylate by the process given by Devi et al [8]. The copolymer was prepared in different ratios and the studies were carried out using 1:1 ratio .

2.2.Assay Method of Urease:

Take urease 0.1 ml and add urea solution 0.2 ml and incubate for 15min then add 5 ml of phenol solution and 5 ml of hypochlorite solution mix well and incubate for 30 minand then analysed by spectrophotometer [10].

2.3.Optimization of various coupling conditions:

We have optimized the amino group by gluataraldehyde and hydroxyl group by p-benzoquinone and p-tolyl sulphonyl chloride.We have done covalent coupling by activating both group separately and also

by simultaneous activation of both groups. The activation agent concentration was checked by varying glutaraldehyde concentration from 1-5 % v/v ,p-benzoquinone 0.5-2.0 gm and p-tolyl sulphonyl chloride from 0.1-0.5 gm . The effect of activation time was checked by varying the time from 2-12 hrs. The effect of pH on coupling was also checked for all the cases by varying the pH from 3-10. The amount of enzyme coupled was also checked by varying the concentration from 0.5 mg-3.0 mg. The enzyme coupling time was checked between 4-20 hrs. We have carried out hydrolysis urea using these covalenty found and free enzyme . We have varied urea concentration from 1-5 % . We have also checked the effect of enzyme loading on urea hydrolysis by varying enzyme concentration from 1-4 mg . More over the effect of pH on urea hydrolysis was also checked.

3.0.Result and Discussion :

The activation agent concentration was found to be better in case of p-tolyl sulphonyl chloride i.e. 0.2 mg/gm of support compared to 1.0 mg/ gm of support . Moreover the coupled enzyme was also found to be more in case of p-tolyl sulphonyl chloride i.e.43 micro gram /gm of support compared to 30 microgram /gm of support in case of p-benzoquinone. Hence p-tolyl sulphonyl chloride was taken for further studies. The amino group was activated by glutaraldehdye and the optimum concentration was found to be 15 ml 3%w/v with coupled enzyme 63 mirogram /gm of support. The activation agent coupling time was found to be 3hrs for p-tolyl sulphonyl chloride , 4 hrs for glutaraldehdye and 5 hrs for p-benzoquinone. While enzyme coupling time was found to be 6,13,10 for p-tolyl sulphonyl chloride , p-benzoquinone , glutaraldehyde. The coupling pH was found to be 7.5 in all cases We have carried out simulatenous activation of both the amino and hydroxyl group and with was found that when first amino group is activated followed by hydroxyl group the enzyme coupling was more i.e. 156 micro gm / gm of support but when the reversed by checked the enzyme coupled was found to be just 18 microgram /gm of support (**Table 1).** Urea hydrolysis was carried out using this system and it was found that with increase in urea concentration the hydrolysis increased but covalently bound enzyme showed more hydrolysis compared to free enzyme **(Table 2)**. The loading was checked and it was found that with the increase with enzyme concentration the hydrolysis increased but there was no significant increase after 3 mg enzyme loading **(Table 3)**. The optimum pH for hydrolysis was found to be 7.0 . **(Table 4)**.

4.0.Conclusion:

Urease enzyme was covalently bound to Acrylamide –co-2 hydroxy ethyl methaacrylate and various covalent coupling condition were optimized and we have observed some interesting results. The urea hydrolysis was carried out using this immobilized system and it was found that covalently bound enzyme showed better results compared to free enzyme in batch process and maximum of 93 % hydrolysis was observed in case of covalently bound enzyme compared to 77 % in case of free enzyme.

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Legends to Tables :

1. Optimum conditions for covalent coupling of urease on Acrylamide-co-2-hydroxyethylmethaacrylate..
2. Effect of urea concentration on hydrolysis of urea using free and covalently bound enzyme.
3. Effect of enzyme loading on urea hydrolysis.
4. Effect of pH on urea hydrolysis using free and covalently bound enzyme.

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Table 1 :

Coupling conditions	***Glutaraldehyde***	***p-tolyl sulphonyl chloride***	***p-Benzoquinone***	***Glutaraldehyde +p-tolyl sulphonyl chloride***	***p-tolyl sulphonyl chloride + Glutaraldehyde***
Activation agent concentration	*15ml 3%w/v*	*0.2 mg/gm of support*	*1.0 mg/gm of support*	*15ml 3%w/v +0.2 mg/gm of support*	*0.2 mg/gm of support + 15ml 3%w/v*
Activation agent coupling time	*4*	*3*	*5*	*4+3*	*3+4*
Covalently bound enzyme	*63 µgm /gm of support*	*43 µgm /gm of support*	*30 µgm /gm of support*	*156 µgm /gm of support*	*18 µgm /gm of support*
Coupling time	*10*	*6*	*13*	*8*	*8*
Coupling pH	*7.5*	*7.5*	*7.5*	*7.5*	*7.5*

Table 2:

Urea concentration (%)	***Relative hydrolysis (%)***	
	Free enzyme	***Covalently bound enzyme***
1	*23*	*46*
2	*52*	*69*
3	*73*	*89*
4	*76*	*91*
5	*77*	*93*

Table 3:

Enzyme concentration (mg)	*Relative hydrolysis (%)*	
	Free enzyme	*Covalently bound enzyme*
1	*25*	*49*
2	*45*	*63*
3	*73*	*89*
4	*76*	*90*
5	*79*	*92*

Table 4:

pH	*Relative hydrolysis (%)*	
	Free enzyme	*Covalently bound enzyme*
3	*16*	*26*
4	*35*	*49*
5	*51*	*62*
6	*62*	*84*
7	*79*	*92*
8	*68*	*78*
9	*46*	*65*
10	*34*	*52*

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